ASSESSMENT OF UTILITY OF URINARY TWEAK (TNF like WEAK inducer of apoptosis) AS A MARKER OF LUPUS NEPHRITIS IN CHILDREN WITH SYSTEMIC LUPUS ERYTHEMATOSUS

PROSPECTIVE OBSERVATIONAL STUDY

A dissertation submitted in partial fulfilment of the rules and regulations for MD Paediatrics examination of the Tamil Nadu Dr.M.G.R Medical University, Chennai, to be held in April 2016

CERTIFICATE

This is to certify that the dissertation entitled, **ASSESSMENT OF UTILITY OF URINARY TWEAK AS A MARKER OF LUPUS NEPHRITIS IN CHILDREN WITH SYSTEMIC LUPUS ERYTHEMATOSUS"** is a bonafide work done by Dr.MuniyaThokchomtowards the partial fulfilment of the rules and regulations for MD Paediatrics degree examination of the Tamil Nadu Dr.M.G.R Medical University, to be conducted in April 2016

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DECLARATION

This is to declare that this dissertation titled, **"ASSESSMENT OF UTILITY OF URINARY TWEAK AS A MARKER OF LUPUS NEPHRITIS IN CHILDREN WITH SYSTEMIC LUPUS ERYTHEMATOSUS"** is my original work done in the partial fulfilment of the rules and regulations for MD Paediatrics examination of the Tamil Nadu Dr.M.G.R Medical University, Chennai to be held in April 2016

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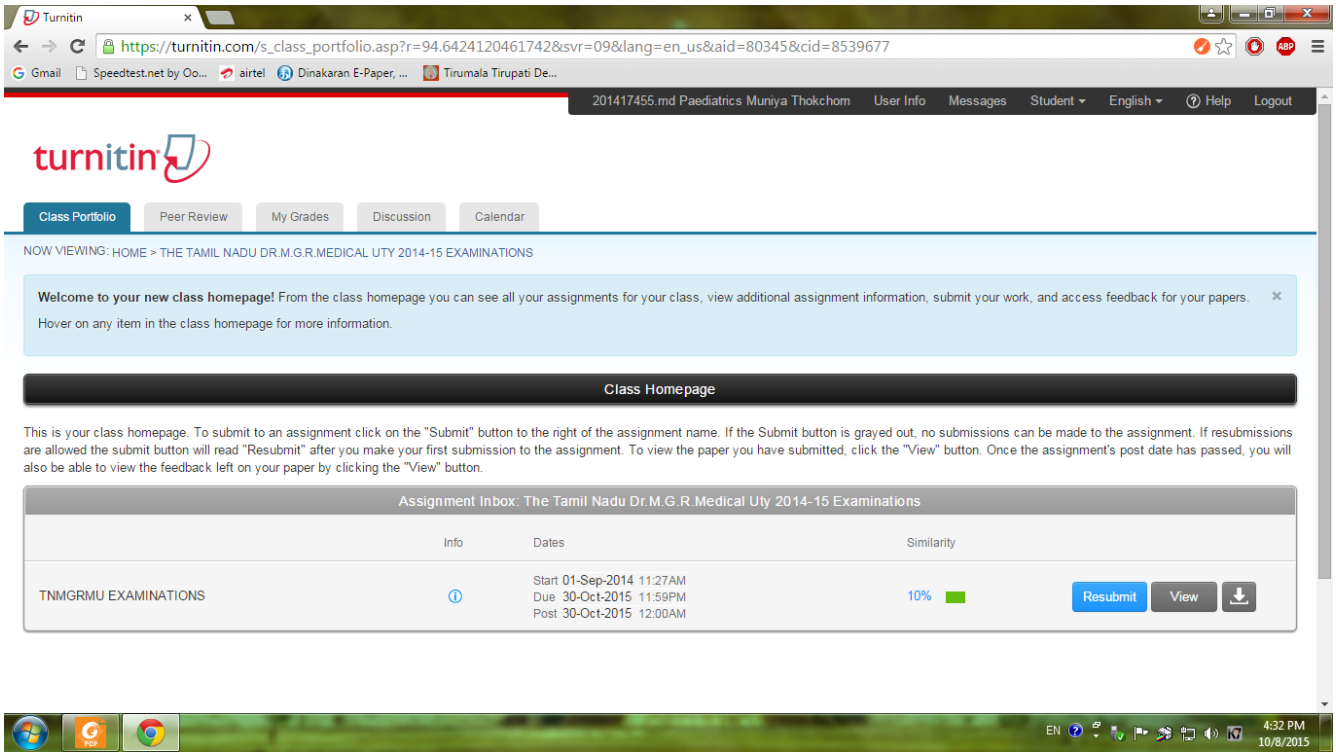
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Dear Dr. Muniya Thokchom,

I enclose the following documents:-

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With best wishes,

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ABSTRACT

TITLE OF THE ABSTRACT: ASSESSMENT OF UTILITY OF URINARY TWEAK AS A MARKER OF LUPUS NEPHRITIS IN CHILDREN WITH SYSTEMIC LUPUS ERYTHEMATOSUS

DEPARTMENT: Child health

NAME OF THE CANDIDATE: MuniyaThokchom

DEGREE AND SUBJECT: MD Paediatrics

NAME OF THE GUIDE: Dr.IndiraAgarwal

KEY WORDS: Urinary TWEAK,biomarker,Systemic lupus erythematoses(SLE)

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OBJECTIVES:

This study was planned to assess the clinical utility of TWEAK in the care and the management of patients with lupus nephritis in children with systemic lupus erythematosus. The primary objective was to assess the correlation of urinary TWEAK level with renal SLEDAI score and also to assess the ability of urinary TWEAK levels to differentiate the class of lupus nephritis as per the histopathological findings (renal biopsy report).

METHODS:

The prospective study was conducted over a period of 11 months (September 2014 – October 2015) in the department of Child Health, Christian Medical College, Vellore, a tertiary care centre in South India. Children with SLE who fulfilled the inclusion criteria were further subdivided based on biopsy report and renal SLEDAI scores into the following groups:SLE with lupus nephritis, SLE without lupus nephritis,disease controls which included children with other autoimmune and renal disease and healthy controls which included healthy children with normal baseline parameters. The primary outcome assessed was the urinary TWEAK levels which was done using the TWEAK ELISA ,which is a quantitative competitive immunoassay .Data analysis was done by means of student's t test, the chi – square test or Mann- Whitney U test, as appropriate. Pearson's correlation with Anova's statistical methods was used to ascertain the significance of statistical correlation.

RESULTS

1) The mean level of Urinary Tweak levels was 3.49 ± 2.29 ng/ml, ranging from 0.02 to 10.35 ng/ml. The urinary TWEAK levels were highest in the SLE without nephritis group, closely followed by the autoimmune /renal disease group. The SLE nephritis group had lower values, but was higher than the healthy Controls. However, the difference between these groups was not statistically significant. (p value = 0.888). Higher renal SLEDAI score had a higher median value of urinary TWEAK. However the values were not statistically significant (p value 0.174).Maximum levels of TWEAK levels were seen in Class III followed by Class II and then by IV lupus nephritis. The difference was not statistically significant (p = 0.174). There was mild positive correlation between TWEAK levels and anti ds DNA, and urine protein creatinine ratio. The difference was not statistically significant($p > 0.05$) .There was negative correlation between urinary TWEAK levels and serum creatinineand complement C3 and C4 levels but it was not statistically significant ($p > 0.05$).The ROC plot showed an Area under the curve of 0.46. The sensitivity of TWEAK for determining the disease activity was 60.53% and the specificty was found to be 36.11%. The cut off value for determining the active disease was > 2.7 ng/ml.

CONCLUSIONS

Urinary TWEAK levels among paediatric age group could not differentiate lupus nephritis patients from SLE patients without renal involvement. Urinary TWEAK levels were also elevated in children with other Autoimmune /renal diseases hence could not differentiate between SLE and non SLE patients. Urinary TWEAK had a positive correlation with renal SLEDAI score hence may be considered to show promise as a marker of activity in Lupus Nephritis. Urinary TWEAK showed positive correlation with ds DNA and urine protein creatinine ratio and negative correlation with creatinine and complement C3 levels. The TWEAK levels were not able to differentiate between the different Classes of Lupus Nephritis.Urinary TWEAK had a sensitivity of 60.53% and specificty of 36.11% for diagnosing Lupus Nephritis among SLE patients. Hence it may be a better screening test rather than a diagnostic test.

Keywords

TWEAK, SLEDAI

INTRODUCTION

Systemic lupus erythematosus (SLE) is a heterogenous autoimmune disorder affecting mainly women of child bearing age, but around 20% of the cases are seen among children less than 18 years of age (1) . The disease is more active and is associated with rapid progression among children as compared to adult onset SLE (2,3). There is a higher incidence of renal involvement (lupus nephritis) in children worldwide ranging from 20-70% (2–6). Similar higher frequency of renal involvement has been documented in India as well (7–9).

Renal involvement is one of the major factors determining the survival outcome among these children (3,10). The disease has a chronic course with remitting and relapsing period and hence warrants periodic monitoring. The overall prognosis has improved dramatically over the last decade, with the use of immune modulators and steroids but renal involvement continues to be the main cause of morbidity and mortality among these children. About 10-15% of the lupus nephritis patients still progress to end stage renal disease at 5 years of diagnosis (9,11,12). Early diagnosis and appropriate treatment of SLE among children is very important in order to prevent long term morbidity and mortality associated with the disease.

Diagnosis of lupus nephritis is based on several markers like anti ds DNA antibodies, complement levels, urinary indices, etc. However the accuracy indices of these markers are far from satisfactory (13–15). Renal biopsy is the gold standard but it is invasive and has limited application in repetitive testing for monitoring disease progression and

response to therapy. Hence the need for reliable markers which are easy to test, in a lesser invasive (blood) or non-invasive (urine) method which would help to diagnose, prognosticate as well as assess efficacy of therapy and monitor disease progression. Several candidate biomarkers are being evaluated with some of them appearing more promising(16–19). In our study we will be focusing on urinary TWEAK.TNF –like-WEAK – inducer of apoptosis (TWEAK) is a new cytokine which was discovered in 1997 (20). TWEAK is expressed by many inflammatory cells and mediates many pro inflammatory activities through its receptor Fn14 receptor which gets upregulated in injured tissues including kidney (21). Increased expression of TWEAK and FN14 receptor in both glomerular and tubular areas have been reported in patients with lupus nephritis suggesting that TWEAK/Fn14 pathway may contribute to the pathogenesis of lupus nephritis(22). Studies evaluating TWEAK as a marker of lupus nephritis are few but the findings are promising. In their cohort study, Schwartz et al 2006, 2009, found urinary TWEAK levels to be significantly higher in LN-SLE patients than in non-LN SLE patients and other disease- control groups (23,24). Also, TWEAK was better in distinguishing LN-SLE and non LN-SLE patients, than other markers like anti ds DNA antibodies and complements.Perusal of literature which will be briefly summarized later clearly shows urinary TWEAK to be promising biomarker in lupus nephritis. Though some information on this aspect is available, very little is found on TWEAK in children and none from the Indian scenario. Hence this study was planned to assess the clinical utility of TWEAK in the care and the management of patients with lupus nephritis. We hope that urinary TWEAK level may increase the sensitivity and specificity of diagnosis, prognosis, assessing response to therapy and monitoring disease progression.

AIMS AND OBJECTIVES

The objective of the study is to assess the utility of urinary TWEAK as a marker of lupus nephritis in children with Systemic lupus erythematosus.

AIMS:

1) To assess the accuracy of urinary TWEAK levels test as a marker of lupus nephritis amongst all children with newly diagnosed SLE
2) To assess the correlation of renal SLEDAI score with urinary TWEAK level
3) To assess the ability of urinary TWEAK levels to differentiate the class of lupus nephritis as per the histopathological findings (renal biopsy report)

HYPOTHESIS:

1) Urinary TWEAK level will differentiate between newly diagnosed SLE with or without renal involvement.
2) Urinary TWEAK levels correlate with histopathology- based class of lupus nephritis.

LITERATURE REVIEW

INTRODUCTION

Systemic lupus erythematosus (SLE) is a heterogenous autoimmune disorder, involving multiple organs, kidney being the most commonly affected organ. SLE with renal involvement (lupus nephritis) is diagnosed by urinary indices, anti ds DNA antibodies, complements and few other tests. But the accuracy indices are far from satisfactory. Kidney biopsy is the gold standard but it is an invasive procedure and has limited application in repetitive testing, for monitoring of renal involvement. Several newer biomarkers are considered promising. Tumour necrosis factor (TNF) like weak inducer of apoptosis (TWEAK) is one such biomarker which has shown to be a promising biomarker for lupus nephritis.

PATHOGENESIS OF SLE

SLE is an autoimmune disorder which results from loss of self tolerance to nuclear antigens and defect in normal haemostatic mechanism of apoptosis.

Normally during cellular degeneration nuclear components of the dead cells are opsonized by complements or removed by phagocytosis, thus preventing their exposure to extracellular compartments and to immune regulatory cells.

In SLE patient, because of defective apoptosis, the nuclear antigens are exposed to antigen presenting cells which lead to the immunization against the nuclear antigen by the plasma cells and memory T cells. Also in SLE, there is compromise of elements

which distinguishes self nucleic acids from that of a virus. This leads to activation Toll-like receptors which triggers an antiviral immunity response against self nucleic acids.

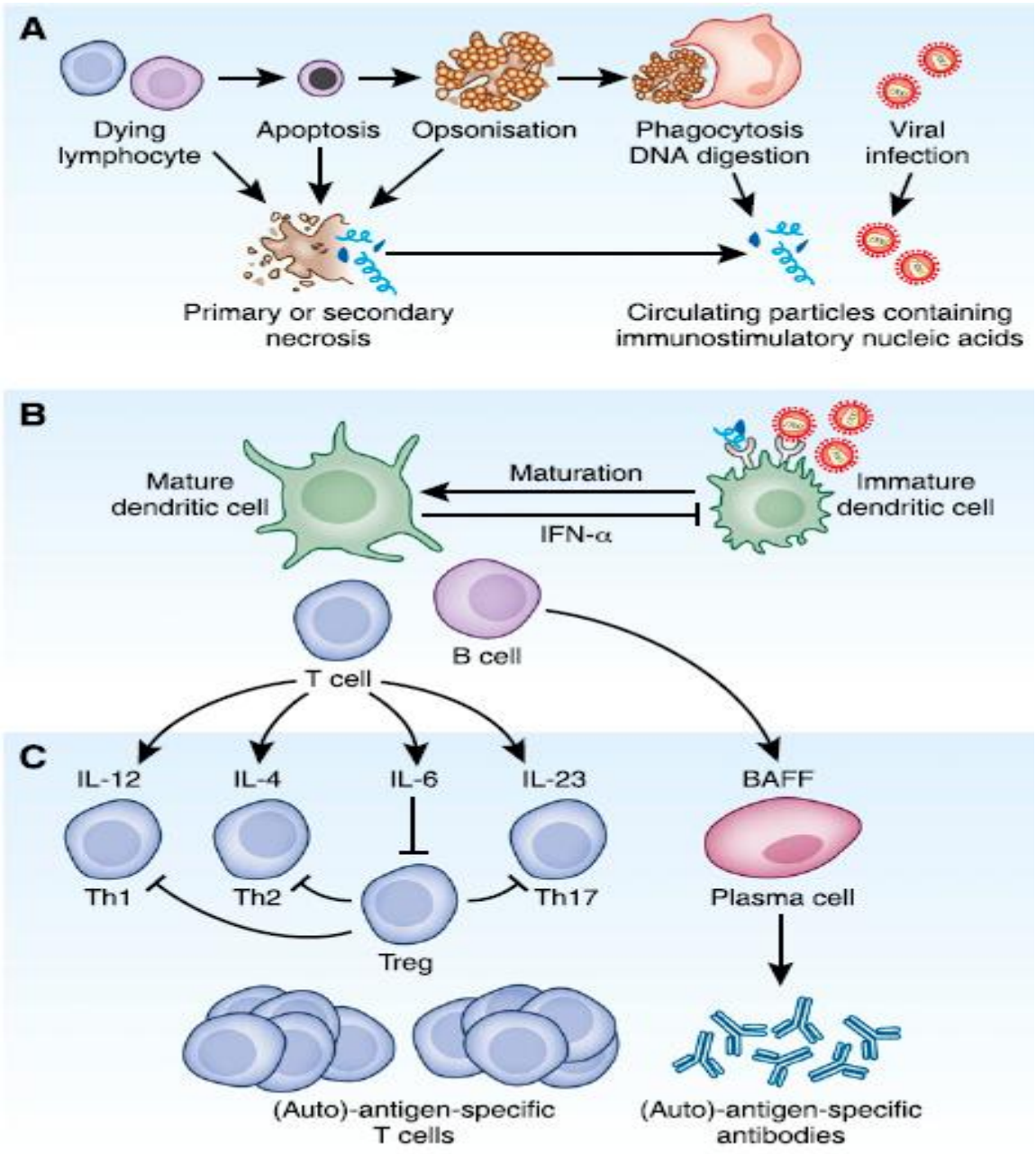


Figure A: Pathogenesis of SLE outside kidney (Adapted from Lech et al, www.jasn.org)

CLASSIFICATION CRITERIA

Due to varied clinical as well as laboratory manifestations of SLE, diagnosis of SLE is made based on classification criteria by the revised American College of Rheumatology (ACR) which are currently used by rheumatologists worldwide.

The criteria for classification were published by Cohen et al in 1971. This criteria was subsequently revised by Tan et al(25) in 1982 to include new serological tests. The currently used ACR criteria was revised in 1997 further to include SLE antibodies (26). According to this classification, a patient is diagnosed to have SLE if 4 or more out of the 11 criteria are present either simultaneously or serially over time.

However this classification is based on adult population and there were no separate classification criteria available for childhood SLE. But studies are available which validated the use of the current 1997 ACR classification criteria for paediatric SLE. One such study done by Ferraz et al(27)evaluated the validity of the 1997 ACR criteria had both 100% sensitivity as well as specificity in diagnosing children with SLE and hence showed that this ACR classification criteria is as accurate when applied to paediatric population as it is among adult SLE (46). However another study done by Tucker et al(28) stated that the clinical profile of paediatric onset SLE are less characteristic as compared to adult disease and also often children present primarily with renal manifestation at onset but lack other criteria for diagnosis of SLE.

DRAWBACK OF THE 1997 ACR CLASSIFICATION CRITERIA

The currently used 1997 ACR classification criteria have not been validated further inspite of the following drawbacks.

1) The criteria has less sensitivity in detecting early disease which might not present with the classical manifestations of SLE since it may take considerable amount of time for the patient to manifest atleast 4 criteria
2) Low complement levels which are seen very commonly at the time of diagnosis or during disease flare is not included in the current 1997 ACR classification criteria
3) This criteria was developed based on studies among the Caucasians and does not take into consideration of ethnicity despite many studies showing variations of clinical manifestations among different ethnic groups.
4) The current 1997 ACR classification criteria was not developed for diagnosing SLE but was actually meant for clinical research purposes.

REVISED 1997 ACR CLASSIFICATION CRITERIA

CRITERIA	DESCRIPTION
1. Malar rash	Raised or flat, fixed erythema over malar prominences, sparing the nasolabial folds
2. Discoid rash	Scaly raised patchy erythema with keratosis
3. Oral ulcers	Painless ulcers
4. Photosensitivity	Rash on sunlight exposure
5. Arthritis	2 or more joints involved with tenderness or joint effusion
6. Renal involvement	Proteinuria of more than 0.5gm/day or urinary dipstick protein of more than 3+ OR Urinary casts
7. Serositis	Pericarditis or pleuritis
8. Haematological involvement	Haemolytic anemia OR Thrombocytopenia with platelet less than 100,000/cu mm OR Leukopenia with counts less than 4000/cu mm OR Lymphopenia with lymphocyte counts less than 1500/cu mm
9. Neurological involvement	Psychosis or seizure
10. Antinuclear antibody	ANA positive
11. Immunological involvement	Anti ds DNA positive OR Anti Smith antibody positive OR Anti phospholipid antibody positive

1. MALAR RASH

Cutaneous manifestations of SLE can be divided into acute and chronic lesion.

Malar rash is an acute lesion which is typically described as erythematous rash present over the malar prominences with sparing of the nasolabial folds and the lesion is exaggerated by ultraviolet light exposure. The rash is classically described as 'butterfly' rash. Studies have shown that malar rash is seen commonly in paediatric age group also (26,29) .

Studies in India have also shown higher incidence of malar among children with SLE. Study done in our own institution by Agarwal et al, Vellore 2009 showed 57.1% of SLE children having malar rash (8).

Other Indian studies like Chandrasekaran et al, Madras1994 showed malar rash in 59% of SLE patients (7) but Singh et al, Chandigarh 2015 showed higher incidence of malar rash (87%) among SLE patients (30)

Figure B Malar rash in paediatric SLE (Adapted from Color Atlas of Paediatric Dermatology)

2. DISCOID RASH

This distinct erythematous scaly plaque is a chronic cutaneous lesion which can progress to keratotic lesions with scarring, mainly on face and scalp. This lesion can lead to alopecia if present on the scalp. However this rash is less commonly seen among paediatric SLE group as compared to adult onset SLE (26,29) and is seen in less than 10% of paediatric SLE cases (31)

Figure C discoid rash causing scarring alopecia (Adapted from DermNet NZ)

3. ORAL ULCERS

These ulcers are described as painless ulcers which are predominantly found on oral and nasopharyngeal cavity and because of their painless nature it can be easily missed during examination unless diagnosis of SLE is specifically considered. These ulcers are found more commonly among paediatric onset SLE as compared to adult onset SLE, more so among Asian paediatric population as compared to Western population (29,32,33). Huang et al 2010

showed that 20 -40 % of SLE children in Asia had oral ulcers at the time of presentation (32). Mondal et al 2010 showed 21% (34) and Singh et al 1997 showed 25% (30)incidence of oral ulcers among Indian children with SLE.

Figure D: Oral ulcer (www.medicalzone.net)

4. PHOTOSENSITIVITY

SLE patients have unusually strong reaction to ultraviolet sunlight exposure which can result in malar rash or even a disease flare. These phenomenon are more common in adult SLE population (47). Photosensitivity was more often found in chronic disease of long duration (29)

5. ARTHRITIS

SLE arthritis are non erosive and mainly involve peripheral joints, 2 or more in numbers, and are characterized by presence of joint tenderness or effusion.

Arthritis is seen commonly in paediatric onset SLE.. In Australia, incidence of arthritis among SLE patients was 76% (35). In other Asian countries, arthritis was less common among paediatric SLE patients. In Taiwan,Lee et al (36) showed only

37% of SLE children having arthritis and in Singapore, by Tan et al (25), arthritis was seen among 56.3% of SLE patients.

However studies in India have showned higher incidence of arthritis among SLE patients. Study in our institution by Agarwal et al 2009 showed 65% of SLE patients having arthritis (8). Similar higher incidence were shown by other Indian studies like Singh et al,1997 which showed 87% involvement (30) and Chandraserakan et al 1994 showing 86.6% of SLE patients with arthritis (7)

6. RENAL INVOLVEMENT

Renal involvement in SLE is described by presence of persistent proteinuria greater than 0.5gm per day or urinary dipstick of 3+ proteins and/or presence of urinary cellular casts including tubular, granular, red blood cells, haemoglobin or mixed. Renal involvement (lupus nephritis) is one of the most common manifestations of SLE seen among paediatric age group (6,30,32,38–42). Similar studies done in India have also showed higher incidence of renal involvement among paediatric onset SLE (7,8,37). Renal disease develop within two years of onset of disease in about 90% of the paediatric SLE population (38). Most often found renal involvement are nephrotic range proteinuria, haematuria, hypertension and renal failure (9,38,39).

The most frequently found histopathological lesion in renal biopsy is class IV lupus nephritis (diffuse proliferative) (9,10,46,47). Renal involvement is one of the major factors determining the survival outcome among paediatric onset SLE (3,10).

Studies done in our institution by Agarwal et al 2009 showed 77.1% renal involvement with histopathological Class II and IV as the most common lesion (8)

whereas other Indian studies like Chandraserakan et al 1994 reported 49.1% (7) and Singh et al 1997 reported 56.2% renal involvement (30).

7. SEROSITIS

Serositis involves inflammation of either the pleura or the pericardium. Patient can presentwith pleuritic chest pain or pleural effusion in pleuritis. They can also present with pericardial rub or pericardial effusion in pericarditis. Serositis manifestation are however, more common among adult onset SLE rather than paediatric SLE (34, 40,50).

8. HAEMATOLOGICAL INVOLVEMENT

Being an autoimmune disease, SLE disease is associated with haematological involvement which is characterized by presence of autoimmune haemolytic anemia (Coomb's positive) associated with reticulocytosis. Anemia can also be due to chronic disease. Thrombocytopenia (platelet count less than 100,000 per cu mm) and leucopenia (total blood count less than 4000 per cu mm) are also commonly seen in SLE patients. They are considered to be due to autoantibodies which are directed against the cell surface antigens. Haematological manifestations are more commonly seen among childhood SLE. (35,40,41). Study done in Singapore by Tan et al, 2015 showed that haematological abnormality (lymphopenia followed by haemolytic anaemia and thrombocytopenia) was the most common manifestation among children with SLE (25). More than 50% of childhood SLE patients present with cytopenia, mild leukopenia being the most common finding (31)

9. NEUROLOGICAL INVOLVEMENT

American College of Rheumatology, 1999 has defined 19 neuropsychiatric syndromes associated with SLE. 25 – 65 % of paediatric SLE children can develop neuropsychiatric symptoms during the course of disease (42,43). One fourth of these children with neuropsychiatric symptoms can have permanent neurological damage (42) . Among the symptoms, headache and psychosis are the most commonly manifested neuropsychiatric symptoms (42,43). Mackie et al in Australia showed only 3% of children with SLE having neuropsychiatric features (35). Similarly other Asian studies also showed lower incidence with only 4.5% neurological involvement in Singapore by Tan et al (25) and 10.5% in Taiwan by Lee et al (36)

However in Indian studies neurological involvement was found to be more common as compared to other countries. Agarwal et al showed 21.4% neuropsychiatric manifestations among SLE patients (8). Comparatively Singh et al showed 23.6% (30) and Chadrasekaran et al showed 27.1% of SLE children with neuropsychiatric manifestions (7).

ACR Neuropsychiatry syndromes seen in SLE (44)

Central Nervous System	**Peripheral Nervous System**
Aseptic meningitis	Guillain Barre syndrome
Headache	Mononeuropathy
Cerebrovascular disease	Polyneuropathy
Seizures	Cranial neuropathy
Movement disorders	Plexopathy
Demyelinating syndrome	Autonomic disorder
Myelopathy	Myasthenia gravis
Mood disorder	
Anxiety	
Psychosis	
Cognitive dysfunction	
Acute confusional state	

10.ANTINUCLEAR ANTIBODY

Positive antinuclear antibody demonstrated by ELISA or immunofluorescence assay at any time during the disease course is seen in majority of the SLE patients. ANA positivity had sensitivity rate of 95 to 98% among paediatric SLE patients (25,43). However it had a low specificity rate of around 36 % as demonstrated by Copple et al, 2011 (45) and also paediatric SLE cases with negative ANA has been reported.

Enriquez et al in 1988 had reported three children with renal biopsy proven lupus nephritis but who were ANA negative (46).

11. IMMUNOLOGICAL INVOLVEMENT

SLE is an autoimmune disorder characterised by production of autoantibodies. So even though ANA positivity is considered as the hallmark of SLE, various other autoantibodies are present which collectively help in a more accurate diagnosis of SLE. So according to the 1997 revised ACR classification criteria, presence of anti ds DNA antibody or anti Smith antibody or antiphospholipid antibodies are predictive of immunological involvement in SLE. A meta analysis study done by Livingston et al, 2012 showed that among these autoantibodies, anti ds DNA antibody was the most commonly found autoantibody in paediatric SLE group with odds ratio of 1.97: 95% confidence interval of 1.31 to 2.96 (47). However Tarr et al, 2015 found that anti-DNA antibody were detected equally among adult as well as paediatric onset SLE (82% in paediatric SLE versus 85% in adult SLE) (48)

Another meta analysis by BIZZARO et al (49) demonstrated that neither anti-ds DNA antibodies was significantly associated with the SLE disease activity ($p = 0.256$) nor was it correlating with renal involvement.

In paediatric SLE, Hiraki et al (50) also found that after ANA, anti-DNA antibodies was the most common autoantibody (84%) but did not find any significant correlation of anti-DNA antibodies with renal disease ($P > .01$)

Esdaile et al found anti ds DNA antibodies to have sensitivity of 50% and specificity of less than 75%. Also it was found to be a poor predictor of disease activity (13,14).

While looking at complements level C3 and C4 in SLE, Birmingham et al found C3 with sensitivity of 27% and specificity of 70% as compared to C4 with sensitivity of 26% and specificity of 49%. During renal flare, only C3 level correlated with renal activity ($p < 0.001$) (51).

In India, Agarwal et al found anti ds DNA antibodies to be positive in 77.1% with low C3, C4 levels in 80% (8) while Chandrasekeran et al showed 92.3% ds DNA antibodies positivity with 50% low C3 among paediatric SLE cases (7)

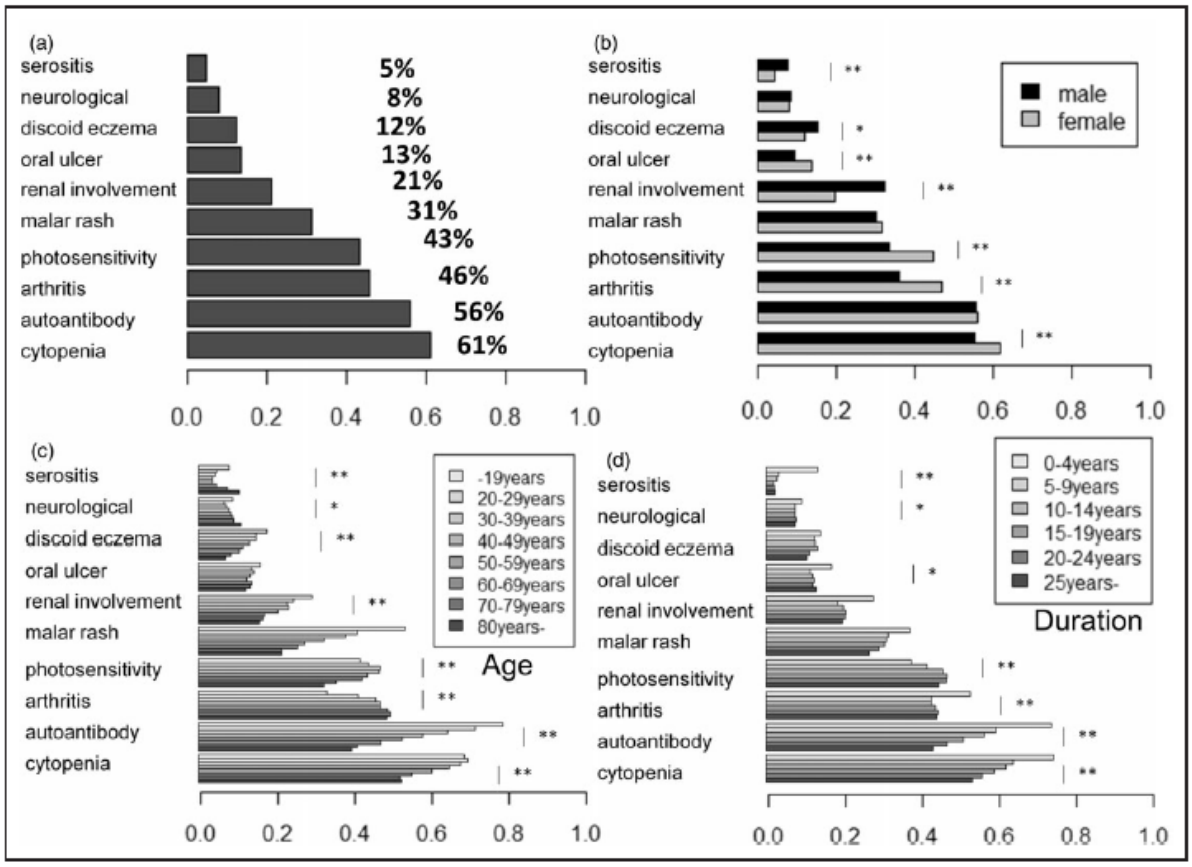


Figure E : Distribution of SLE features (Adapted from Terao et al, Lupus 2014)

BIOMARKERS OF SLE

BIOMARKERS	INDICATORS
Anti ds DNA	Disease flare Renal involvement
C3 and C4	Disease flare Renal involvement
Anti C1q	Disease activity Lupus nephritis
Urinary TWEAK	Renal flare
Urine MCP-1	Renal flare
Anti C1q and anti ds DNA	Renal flare
Anti phospholipid and anti ribosomal P protein	Neuropsychiatric SLE

ASSESSMENT OF DISEASE ACTIVITY

Systemic lupus erythematosus is a disease with remitting and relapsing course. The disease flare is diagnosed by using both clinical and laboratory features. Different tools for assessment of disease activity are available. Some of the commonly used disease activity indices are the following:

1) SLE disease activity index (SLEDAI)
2) British Isles lupus assessment group (BILAG)
3) Systemic lupus activity measure (SLAM)
4) European consensus lupus activity measurement (ECLAM)

Of the indices described, SLEDAI and SLAM are found to be the most user-friendly (52). SLEDAI score gives more attention to renal disease when compared to SLAM (16 points versus 8 points). Hence it is more helpful in assessing SLE patients with renal involvement (53). Renal SLEDAI includes only 4 parameters which are used to assess renal disease activity – proteinuria, haematuria, pyuria and urinary casts. So far renal SLEDAI has been found to be best for measuring lupus nephritis activity among the currently available indices (54).

SLEDAI score has been added in the **Annexure I**

EPIDEMIOLOGY

INCIDENCE AND PREVALENCE WORLDWIDE

About 15-20% of systemic lupus erythematosus cases are seen among children less than 18 years of age (1,32,33,55). Incidence of SLE among children differs according to the ethinicity with higher frequency of incidence in Asian, Hispanic, African American, Australian aboriginals and Native American as compared to Caucasians. Incidence varies widely across the countries of the world ranging from 0.36 to 2.5 per 100,000 in countries like America, United Kingdom and Australia to about 31/100,000 per year among Asian countries (3,35,43). Prevalence of childhood onset SLE varies from 1.89 to 25.7 per 100,000 (56)

TRENDS IN SOUTH EAST ASIA

Studies in South East Asian countries have shown a higher prevalence of childhood SLE as compared to Western countries. In Taiwan the prevalence rate was 6.3 per

100,000 (57) while another study in Singapore showed a prevalence of 14.2 per 100,000 children (25). A study done by Huang et al, 2010 showed prevalence of paediatric onset SLE as 6.3 to 19.3 per 100,000 children in Asia (32)

INCIDENCE AND PREVALENCE IN INDIA

There is paucity of reports in India regarding the incidence and prevalence of childhood onset SLE. Study done in Eastern India showed that 3.9% of all paediatric rheumatological cases were SLE patients (37). Another study done in North India showed a point prevalence of 3.2 per 100,000 (58)

SEX DISTRIBUTION

Like in adult, females are more commonly affected in SLE among children with male : female ratio ranging from 1: 2 to 1: 7 (7,8,32,37,43). Huang JL et al, 2004 found that the prevalence among girls was 6.2 times higher than those among boys (57). A study done in our own institution by Agarwal et al, 2009 showed male : female ratio of 1:6 (8)

AGE DISTRIBUTION

Most of the childhood SLE have disease onset between 5 to 16 years of age (32,43,48). In Agarwal et al study, 2009 the mean age group of SLE was found to be 10.5 year (range 4 to 15 years) and in Singh et al (30) it was 10 years. Female patients are found to develop disease onset at a younger age than compared to male counterpart (29)

DIFFERENCE BETWEEN ADULT AND PAEDIATRIC ONSET SLE

The disease is more active and is associated with rapid progression among children as compared to adult-onset SLE (2,5,28,55,59). Clinical manifestations also differ between adult and childhood onset SLE. Features like fever, mucocutaneous lesions, renal ,neurological, haematological problems and polyarthritis were more common in children (5,33,59,60). Childhood SLE may have different autoantibody profiles (increased anti–dsDNA and anticardiolipin antibody, less rheumatoid factor), and more disease activity than adult-onset SLE

RENAL INVOLVEMENT IN PAEDIATRIC SLE

There is a higher incidence of renal involvement (lupus nephritis) in children worldwide ranging from 20 to 70% (6, 30, 32, 40–42). Similar higher frequency of renal involvement has been seen in India as well (7,8,37).

Renal involvement is one of the major factors determining the survival outcome among these children (2,3,10,33,59). Renal involvement is most commonly manifested by nephrotic range proteinuria, haematuria, hypertension and high creatinine(9,12,61).

In Lee et al study, proteinuria was seen in 51.3%, haematuria in 44.4% and cellular cast in 10.5% of the paediatric SLE cases (36). Tan et al in Singapore found haematuria more commonly (50%) followed by proteinuria (39.1%) (25).

Singh et al study showed proteinuria in 94.4%, haematuria in 62.5%, hypertension in 37.5% and high creatinine in 19.4% among paediatric SLE (30).

Most frequently seen histological finding in renal biopsy among lupus nephritis is class

IV nephritis (diffuse proliferative) (9,12,36,39).

When renal parameters were compared with the class of lupus nephritis, it was found that proteinuria, haematuria, hypertension and elevated creatinine level correlated with higher classification of lupus nephritis. Studies in paediatric age group also showed similar findings with Class IV lupus nephritis group being found to have higher incidence of nephrotic range proteinuria ($p < 0.05$) as compared to other classes in Yang et al study (62). Agarwal et al also showed Class IV lupus nephritis to be more commonly associated with high creatinine (83.3%), haematuria (66.6%), hypertension (61.5%) and proteinuria (58.1%) (8)

LUPUS NEPHRITIS

Renal involvement (Lupus nephritis) is one the most common clinical manifestations of paediatric SLE. Incidence of lupus nephritis among paediatric SLE ranges from 20 to 70% (6, 30, 32, 40–42). Similar higher frequency has been documented in India as well (36 - 39). Lupus nephritis is more active in childhood SLE and is one of the major factor determining the survival outcome among these children with around 10-20% of these children developing end stage renal disease eventually (2,3,10,33,59).

PATHOGENESIS OF LUPUS NEPHRITIS

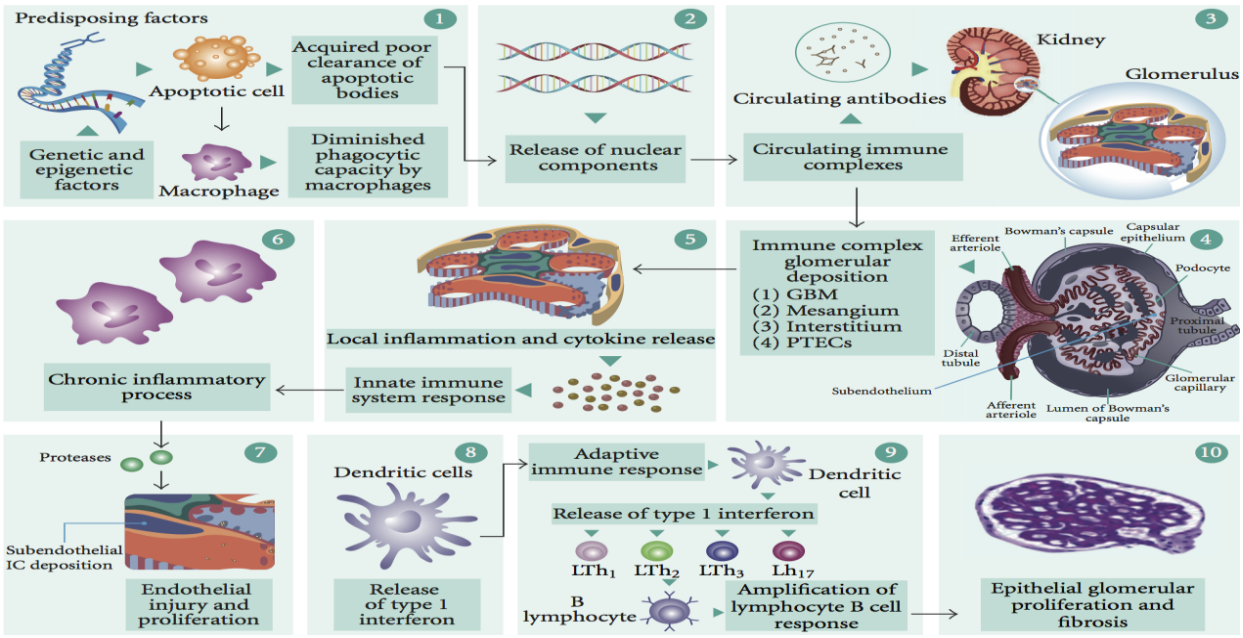


Figure F :Pathogenesis of Lupus nephritis (Adapted from Marc C, Rheumatology)

PROPOSED MECHANISM OF TISSUE INJURY IN LUPUS NEPHRITIS

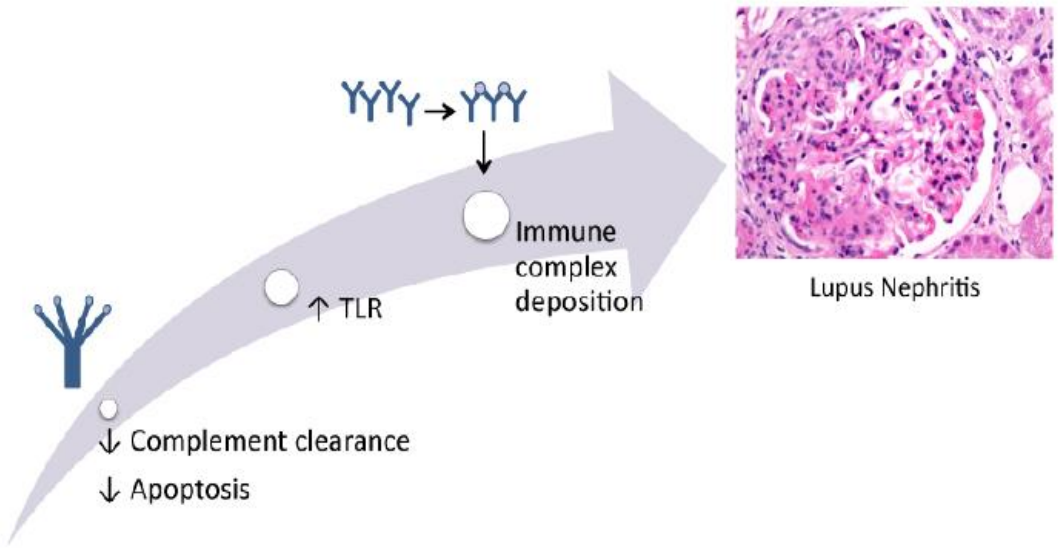


Figure G : Proposed mechanism of immune complex deposition in Lupus nephritis (Adapted from J Clin Cell Immuno, 2014)

DEPOSITION OF IMMUNE COMPLEXES

Deposition of autoantibodies as immune complexes is a pre requisite for development of lupus nephritis (63). Anti ds DNA antibodies are the most commonly linked autoantibody in development of lupus nephritis (64). The exact mechanism of immune complexes deposition in the glomerulus is not known but there are few postulations. Three theories have been postulated so far:

1) Deposition of circulating preformed serum immune complexes
2) In situ binding of the autoantibodies to endogenous glomerular antigens
3) Direct binding of autoantibodies to endogenous glomerular antigens like apoptotic DNA/ nucleosomes ('planted antigen') (63,65–67).

ROLE OF COMPLEMENT IN LUPUS NEPHRITIS

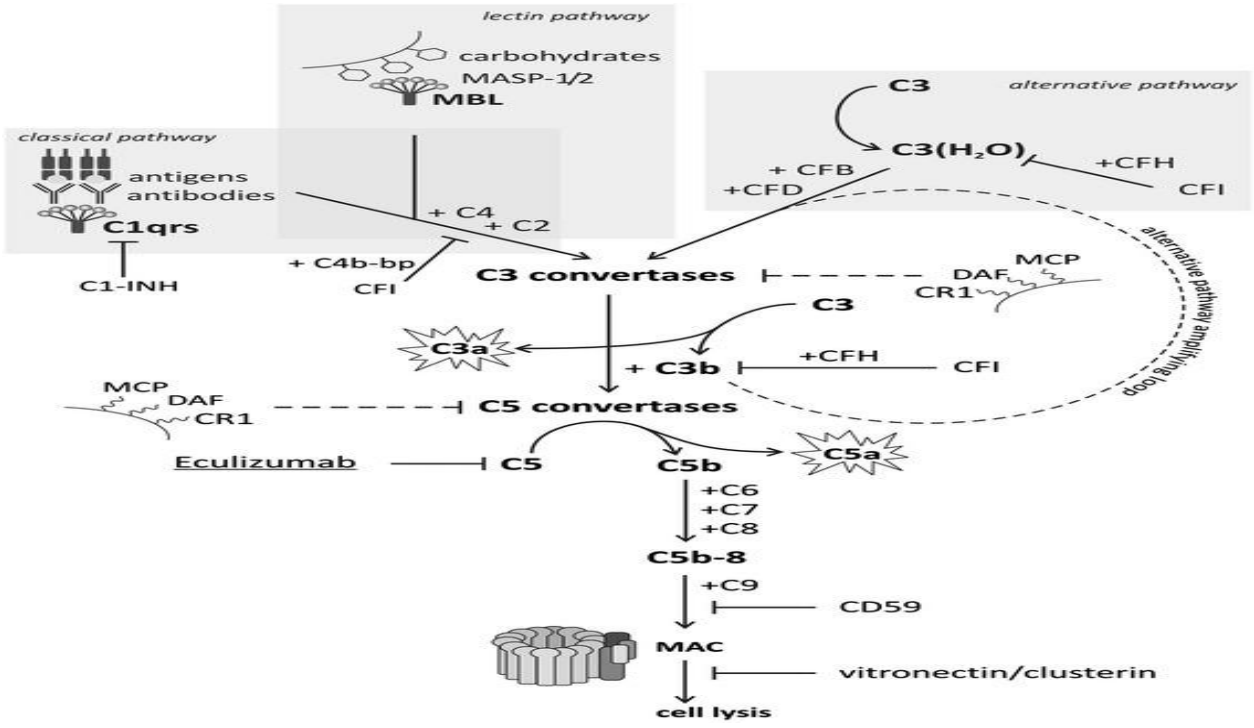


Figure H : Classical complement activation pathway in Lupus nephritis (Adapted from Arch ImmunolTherExp, 2013)

Complement activation has a significant role in the pathogenesis of lupus nephritis. Both classical and alternative pathways are activated in lupus nephritis (66,68,69). Initially classical complement pathway is activated by C1 which binds to the Fc part of the immune complexes and results in release of C3 convertase .Simultaneously the alternative pathway is also constantly activated which results in further generation of C3 convertase. C3 convertase cleaves C3 into C3a and C3b opsonin which results in amplification of the activation signals. C3b also generates C5 convertase, which finally leads to lysis of the target cells (66,68–70).

ROLE OF CYTOKINES IN LUPUS NEPHRITIS

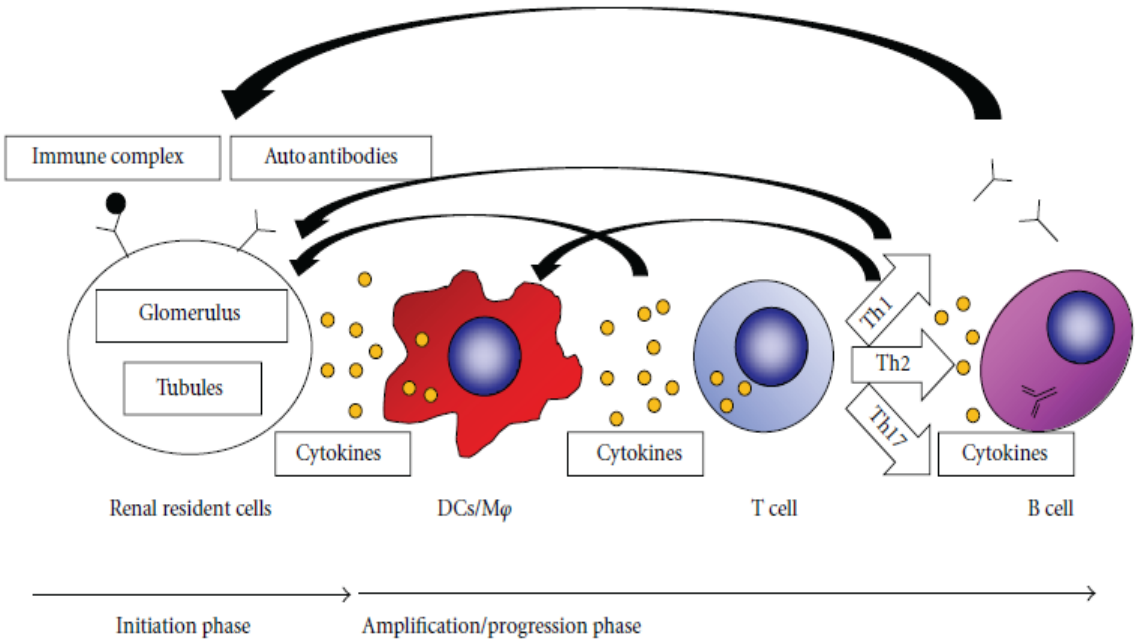


Figure I : Cytokines role in pathogenesis of lupus nephritis (Adapted from Iwata et al, journal of Biomed and biotech, 2011)

CYTOKINES	MAJOR EFFECTS
TNF	Tissue damage
IFN gamma	Tissue damage and inflammation
IL-1	Tissue damage
IL-4	Tissue fibrosis
IL-6	Increased cellularity
IL-8	Leucocyte infiltration
IL-10	Apoptosis induction
IL-18	IFN gamma stimulation
TGFβ	Tissue fibrosis

Immune complexes deposition leads to activation of complement cascade which further causes activation and proliferation of mesangial cells. These activated mesangial cells are responsible for production of various cytokines and chemokines which leads to amplification of the inflammatory processes in the glomerular disease (71). In addition, studies on human cultured mesangial cells have shown anti ds DNA antibodies to induce release of cytokines like interleukin 1β, interleukin 6 and tumour necrosis factor (TNF α) (72). Hence it can now be established that both immune complexes and autoantibodies are responsible for release of cytokines which further amplifies the inflammatory processes seen in lupus nephritis.

CYTOKINES IN LUPUS NEPHRITIS

The main cytokines present in lupus nephritis are the T-helper type 1 cytokines. They produce interleukin -12, interleukin – 18 and interferon gamma. (73)

Overexpression of inerleukin -12 and interleukin – 18 has been observed in glomeruli of mouse as well as human lupus nephritis which resulted in leucocyte infiltration and nephritic renal pathology with proteinuria (74). Interleukin -18 and interleukin -12 inturn causes upregulation of interferon gamma which is responsible for the apoptotic destruction renal cells seen in lupus nephritis (75)

Another cytokine which was discovered recently in 1997 and proven to be associated with the pathogenesis of lupus nephritis belongs to the tumour necrosis superfamily (20). The cytokine TWEAK (tumour necrosis factor weak inducer of apoptosis) stimulates mesangial cells to secrete proinflammatorychemokines like MCP -1, RANTES (CCL5), CXCL-1, CVCAM-1 which leads to recruitment of activated T cells resulting in the pathogenesis of lupus nephritis (76–78)

CHEMOKINES IN LUPUS NEPHRITIS

Chemokines help in recruitment of inflammatory cells in the kidney. Some of the chemokines are monocyte chemo attractant protein (MCP-1), RANTES (CCL5), CCL4, CXCL10 and macrophage colony stimulating factor. Out of these chemokines, monocyte chemo attractant protein (MCP -1) has been demonstrated to be associated with renal damage in lupus nephritis (73,79).

INFLAMMATORY CELLS

Both B cells and T cells contribute in the pathogenesis of lupus nephritis.

T CELLS IN LUPUS NEPHRITIS

T cells contribute to kidney injury by various mechanisms like cytokine production, recruitment of other inflammatory cells like macrophages and dendritic cells and activation of antibody producing B cells. In fact studies have shown that blockage of T cell activation as well as T cell depletion have reduce the progression of renal damage in mouse models with lupus nephritis (69,80,81).

B CELLS IN LUPUS NEPHRITIS

B cells are primarily responsible for production of plasma cells which inturn produces auto antibodies which are responsible for immune complexes deposition followed by cascade of inflammatory processes seen in lupus nephritis (69,82).

Overall, release of cytokines and chemokines results in influx of inflammatory cells like T cells, B cells and macrophages, ultimately causing renal injury and fibrosis (69).

PATHOLOGY OF LUPUS NEPHRITIS

Glomerular lesions in lupus nephritis have a diverse histo- pathological manifestations and hence the need for a classification system which will help not only in primary patient care but also assist in clinical trials and response to therapy in terms of improvement of renal lesion.

First such classification was made by WHO in 1974 which subsequently underwent modifications in 1982 and 1995. The currently used revised classification was proposed by Renal Pathology Society and International Society of Nephrology in 2003.

Histological examination of minimum of 10 glomeruli is required to exclude any focal lesion. Immunofluorescence study should be done for IgA, IgM, and IgG along with C3 and C1q complement components.

1974 WHO CLASSIFICATION OF LUPUS NEPHRITIS

CLASS I	Normal glomeruli by light microscopy, immunofluorescence, electron microscopy
CLASS II	Purely mesangial disease IIa : normocellularmesangium by light microscopy but mesangial deposits by immunofluorescence and electron microscopy IIb : mesangial hypercellularity with mesangial deposits by immunofluorescence or electron microscopy
CLASS III	Focal proliferative glomerulonephritis (< 50%)
CLASS IV	Diffuse proliferative glomerulonephritis (>/= 50%)
CLASS V	Membranous glomerulonephritis

CLASSIFICATION OF LUPUS NEPHRITIS (based on International Society of Nephrology/ Renal Pathology Society, 2003)

CLASS I LUPUS NEPHRITIS

It is defined as minimalmesangial disease with mesangial immune deposits demonstrated by immunofluorescence

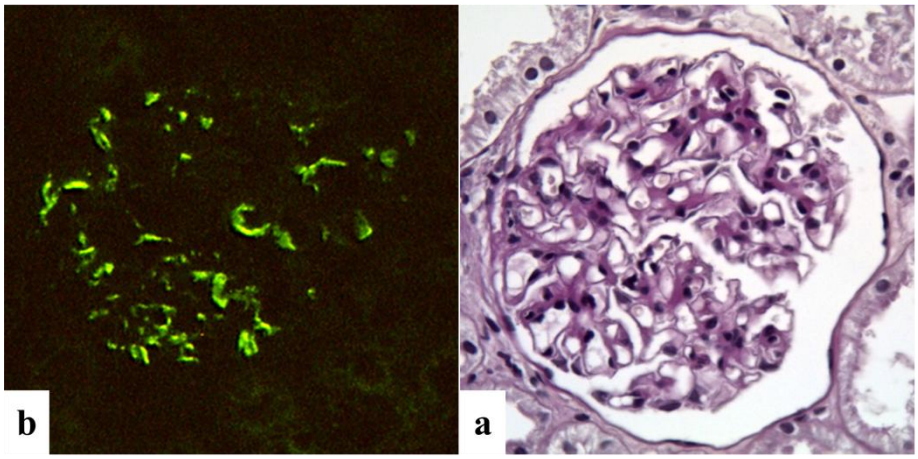


Figure J: Class I lupus nephritis (Adapted from Scientific world journal, 2014)

CLASS II LUPUS NEPHRITIS

Also known as mesangioproliferative lupus nephritis, it is characterized by mesangial hyper cellularity associated with immune deposits in mesangium. Sub epithelial or sub endothelial deposits may be demonstrated by electron microscopy or immunofluorescence .

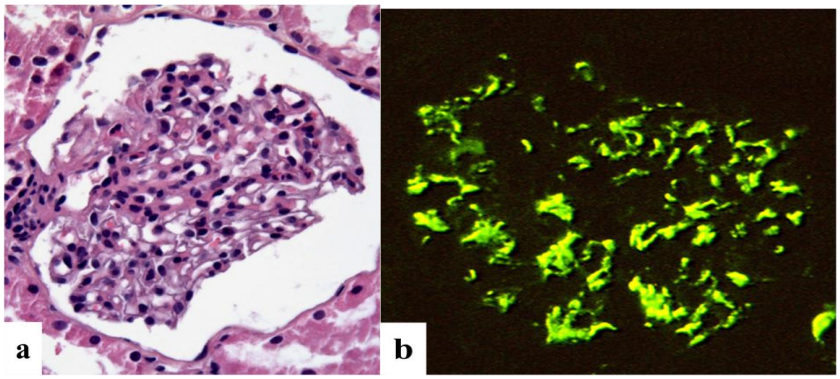


Figure K: Class II Lupus Nephritis (Adapted from Scientific world journal, 2014)

CLASS III LUPUS NEPHRITIS

It is defined as focal lupus nephritis which involves less than 50% of the glomeruli. It was involves description of active or chronic lesions based on the presence of focal proliferation or focal sclerosis respectively.

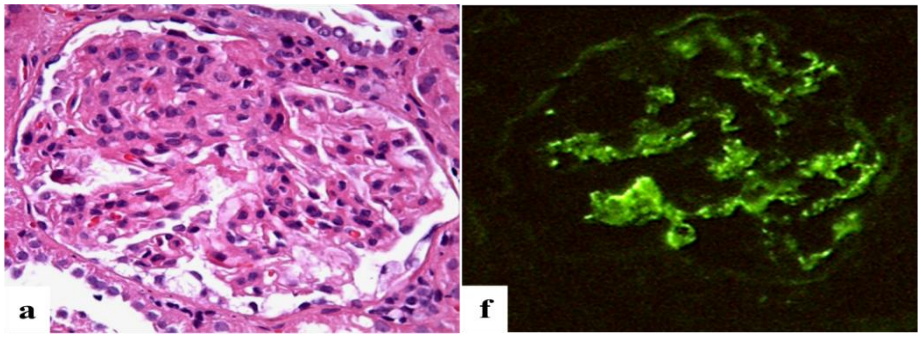


Figure L: Class III Lupus nephritis (Adapted from Scientific world journal, 2014)

CLASS IV LUPUS NEPHRITIS

It is defined as diffuse proliferative lupus nephritis which involves more than 50% of the glomeruli. It is subdivided into segmental and global proliferation. It can also be further subdivided into diffuse segmental (ClassIV-S) when more than 50% of glomeruli show segmental lesions and diffuse global (Class IV – G) when more than 50% of glomeruli shows diffuse proliferation. The description should include proportion of glomeruli which are affected by active or chronic lesions.

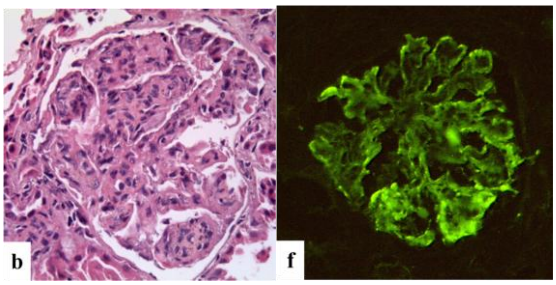


Figure M : Class IV Lupus nephritis (Adapted from Scientific world journal, 2014)

CLASS V LUPUS NEPHRITIS

It is defined as membranous lupus nephritis with segmental or global subepithelial deposits with or without mesangial hypercellularity.

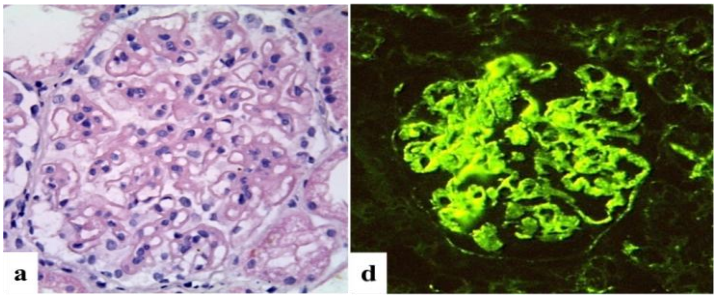


Figure N : Class V Lupus nephritis (Adapted from Scientific world journal, 2014)

CLASS VI LUPUS NEPHRITIS

It is defined as advanced sclerotic lupus nephritis with more than 90% of the glomeruli undergone glomerulosclerotic changes and without any evidence of active disease.

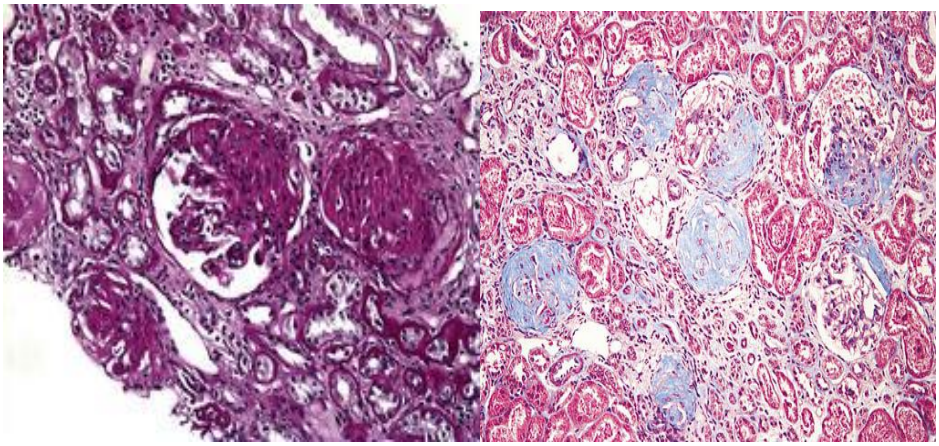


Figure O : Class VI Lupus nephritis (Adapted from Hepinstall's pathology of kidneys)

ABBREVIATED ISN/RPS Classification of Lupus nephritis

CLASS I	Minimal mesangial lupus nephritis
CLASS II	Mesangioproliferative lupus nephritis
CLASS III	Focal lupus nephritis
CLASS IV	Diffuse segmental or Global lupus nephritis
CLASS V	Membranous lupus nephritis

The most common histological lesion found in paediatric SLE is Class IV (diffuse proliferative) followed by Class III (focal lesion) and Class II (mesangioproliferative) (10,38,39,62). Indian studies also showed similar predominance of class IV lupus nephritis. Singh et al 2015 study had Class IV in 66% followed by Class II in 18.9% of patients (83) . Hari et al 2009 also showed higher incidence of Class IV (48.1%) followed by Class II (18.5%) (9). However Agarwal et al 2009 showed equal distribution of Class IV and Class II in 44.4% followed by Class III in 4.3% of paediatric lupus nephritis cases (8).

RENAL BIOPSY IN LUPUS NEPHRITIS

Renal biopsy is the gold standard for diagnosis of lupus nephritis. Similar clinical features may be seen in patients with different classes of lupus nephritis. This is particularly important since aggressive treatment with harmful side effects are generally reserve for more progressive renal disease. Therefore renal biopsy should be done earlier during the disease course in order to plan early treatment (84). Studies have shown that renal damage in lupus nephritis over the last decade have decreased due to early diagnosis and timely treatment (85)Faurschou et al, 2006(86) study showed that

delay in renal biopsy following the onset of nephritis constituted one of the risk factors for end stage renal disease (86) .However lupus nephritis has a chronic course with remitting-relapsing period and hence warrants periodic monitoring. Repeat renal biopsy especially in children has limited application. Also there are no clear consensuses available for repeat renal biopsy in lupus nephritis. The most common reason for repeat renal biopsy is when the patient has worsening renal functions like persistent proteinuria, raising creatinine or worsening urinary sediments. The other most common reason is when the patient does not respond during induction therapy (87)

BIOMARKERS IN LUPUS NEPHRITIS

DIAGNOSIS	Anti ds DNA antibodies
	C4d – erythrocyte/platelet bound
ACTIVITY	Anti ds DNA antibodies
	Complements : C3,C3a,c3d, C4, C5a, C4d
	Cytokines : IL-6, IL-12, IL-10,IL-15,IL-18, IFN gamma, TNF α
	Endothelial activation markers : sICAM, sVCAM
	Acute phase proteins : CRP, ferritin
RENAL INVOLVEMENT	Anti ds DNA antibodies Anti C1q Urinary VCAM and MCP-1

	Urinary NGAL Urinary TWEAK (tumor necrosis factor–like weak inducer of apoptosis)

As mentioned earlier, repeat renal biopsy for monitoring lupus nephritis disease progression and response to therapy is not applicable especially in paediatric group of patients. Also many studies have shown that the currently available markers for diagnosis of lupus nephritis like anti ds DNA, complement levels and urinary indices have varying accuracy levels and their levels do not correlate well with the disease activity(13–15).

Several biomarkers are being evaluated with some of them showing promising results. A summary of the markers evaluated can be found in the reviews of Shwartz et al 2007 (88), Manmohan &Madaio 2010 (89), Herst et al 2012 (90), Bennet& Brunner 2013 (91).

In the present study our focus in on urinary TWEAK (TNF like WEAK inducer of apoptosis)

TWEAK (TNF like WEAK inducer of apoptosis)

TWEAK (TNF like WEAK inducer of apoptosis) is a new cytokine which was discovered in 1997 and belongs to TNF superfamily (20). TWEAK is expressed as a soluble cytokine by various inflammatory cells like leukocytes, macrophages, dendritic

cells and natural killer cells. It has been found to be involved in proinflammatory responses, vascular changes, cellular proliferation, apoptosis and fibrosis (21).

TWEAK/Fn14 receptor expression in kidney

Fn14 is the receptor for TWEAK is expressed on various cell types like epithelial, mesenchymal and endothelial cells. In kidneys, it is expressed on mesangial cells, podocytes and tubular cells and also by the infiltrating leucocytes(76). Normally Fn14 is expressed at low levels but is readily up regulated in disease tissues and responsible for the inflammation and injury. Fn14 expression are increased by the proinflammatory cytokines like TNF α. IFN gamma and also by growth factors (76,92).

TWEAK ACTIONS OF KIDNEY CELLS

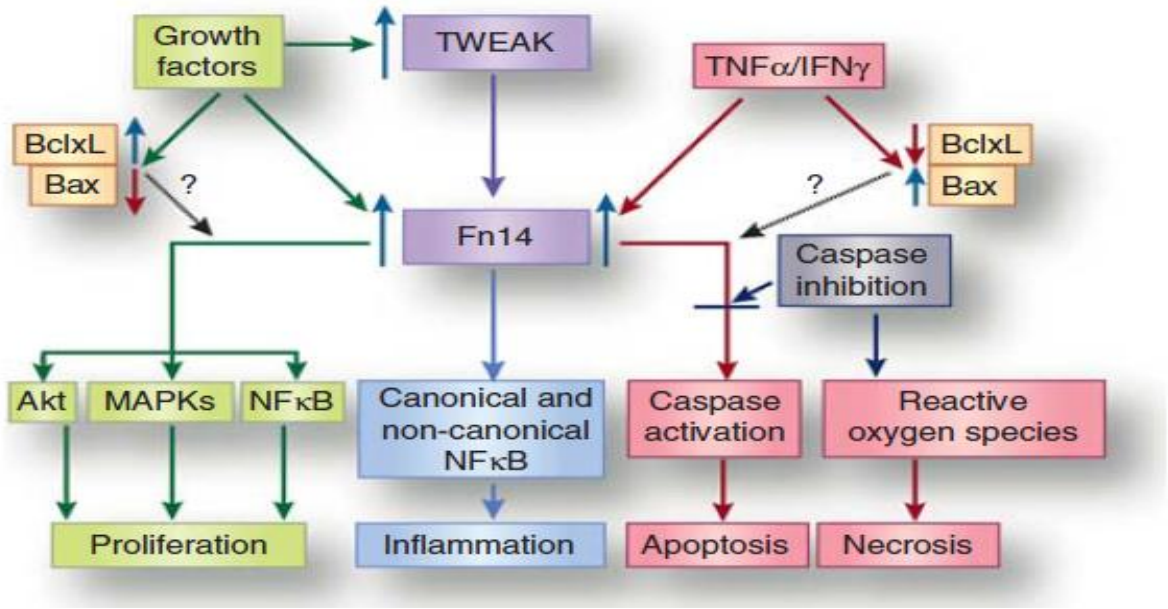


Figure P : TWEAK actions on renal cells (Adapted from Kidney International, 2011)

TWEAK ACTION ON RENAL CELLS

Cell type	Action
Tubular cells	Proliferation Inflammation
Mesangial cells	Proliferation Inflammation Apoptosis
Podocytes	Proliferation Inflammation

PROINFLAMMATORY EFFECTS

The proinflammatory action of TWEAK includes release of various cytokines and chemokines which promote inflammatory cells infiltration followed by renal tissue damage. In fact studies have shown that TWEAK/Fn14 pathway deficiency or blockade is associated with decreased disease activity in renal disease models (87–89).

VASCULATURE EFFECTS

TWEAK has been shown to induce angiogenesis by activation of endothelial cells (93). However it is yet to be proven whether TWEAK has a role on the vascular permeability of the glomerular capillaries resulting proteinuria.

EFFECTS ON MESANGIAL CELL PROLIFERATION

Studies have shown TWEAK stimulating mesangial cell proliferation both in vivo and in vitro (94). Mesangial hyperplasia is one of the pathognomic features of lupus nephritis.

EFFECTS ON APOPTOSIS

Renal injury and atrophy are seen in progressive lupus nephritis. TWEAK along with IFN gamma has been shown to induce apoptosis of mesangial cells (95). This statement has also been complemented by the study that TWEAK/Fn14 pathway deficiency or blockade results in decreased renal inflammation (96).

Recent studies on TWEAK have shown it to be a reliable marker for lupus nephritis in adult SLE patients. Studies have also show that TWEAK levels correlate with disease flare**.** The first study on TWEAK was done by Schwartz et al, 2009 (24) This multicentre cohort study was done on lupus nephritis with active disease and SLE without renal involvement. They also included four groups as control – healthy controls, other renal diseases due to hypertension or diabetes, rheumatoid arthritis and osteoarthritis patients. This study was primarily done among adult population with age ranging from 28 to 67 years of age. Urinary TWEAK levels were found to be elevated among lupus nephritis patients as compared to SLE patients without nephritis with median urinary TWEAK level of 12.54 (5.00 to19.38) pg/mgCr in active lupus nephritis versus 5.02(1.94 to 9.11) pg/mgCr ($P < 0.001$) in SLE patients without lupus nephritis. Urinary TWEAK levels were found to be significantly lower in the control groups – SLE without nephritis ($p = 0.005$), rheumatoid arthritis group ($p = 0.013$) and healthy control ($p = 0.003$) when compared to lupus nephritis group.

TWEAK levels were shown to be a better predictor of renal involvement than anti ds DNA or complement levels with odds ratio of 7.36 (95% CI = 2.25 to 24.07, p value = 0.001). They further showed that urinary TWEAK level peaked during lupus nephritis disease flare (p value < 0.05) (24).

Another study done by Xuejing et al, 2012 compared level of TWEAK among two groups – active lupus nephritis versus non active lupus nephritis with age group ranging from 14 to 53 years. Urinary TWEAK levels among active lupus nephritis were found to be elevated as compared to non – active lupus nephritis patients (10.29 ± 1.81 pg/mgCr versus 2.14 ±0.30 pg/mgCr, P <0.01). They also found significant correlation between elevated urinary TWEAK and disease activity index (correlation coefficient 0.825, p value <0.01). However the urinary TWEAK was not able to differentiate between different classes of lupus nephritis. Moreover the TWEAK levels among healthy controls were higher than those among SLE without nephritis cases. They found urinary TWEAK to have a low sensitivity of 50% and specificity of 90% (97).

Xuejing et al study (97) also did not look into the correlation between levels of urinary TWEAK and different histopathological class of lupus nephritis.

TWEAK is considered to be of renal origin but there were studies which showed lower levels of TWEAK among renal diseases. Kralisch et al (98) and Carrero et al (99) found that serum TWEAK levels were low among renal disease patients on haemodialysis as compared to control patients (p <0.05).

Though TWEAK is detected in both serum and urine, only urinary TWEAK has been found to correlate well with renal pathology in some studies done on adults. There are no reports on TWEAK either on children or among Indians.

Hence this study has been done to assess the utility of urinary TWEAK in identifying renal involvement in children with SLE as well as to see if the TWEAK levels correspond with the disease flare or progression.

METHODOLOGY

SAMPLE AND SETTING

This study was conducted between October 2014 and September 2015 at Paediatrics unit II, CMCH, Vellore. Children presenting with SLE with or without lupus nephritis fulfilling the inclusion criteria were recruited. This included children who attended the Paediatric Rheumatology, Paediatric Nephrology OPD and those admitted in paediatric wards. Healthy children from general OPD who came for general check-up were also recruited as Controls. Recruited children were divided into four groups:

1) Children with lupus nephritis
2) Children with SLE but without lupus nephritis
3) Children with other autoimmune disease and other renal disease

4) Healthy children

The study and research procedures were fully explained to the parents and the patient and those who gave written consent and assent were allowed to participate in the study. The consent was obtained in the regional language that the parent/ patient was conversant **(ANNEXURE II)**

STUDY DESIGN

This is an observational study which was done to assess the utility of urinary TWEAK as a marker of lupus nephritis and correlate its level with the disease activity as indicated by the renal SLEDAI score. Urinary TWEAK level was also correlated with other pre-existing indicators of lupus nephritis like anti dsDNA and complements C3, C4 levels.

PARTICIPANTS

INCLUSION CRITERIA

Group I: Children with SLE who were further subdivided based on biopsy report and renal SLEDAI score, into :

Group Ia: SLE with lupus nephritis

Group Ib: SLE without lupus nephritis

Group II : Disease controls which included children with other autoimmune and renal diseases.

Group III : Healthy controls which included healthy children with normal baseline investigation

EXCLUSION CRITERIA

Children whose parents/ legal guardian did not give consent were not included in this study.

All the children meeting the inclusion criteria were enrolled into the study thus minimizing the chances of any selection bias. The urinary TWEAK test was done by the laboratory technicians in the nephrology department who was unaware of the patient groups thus minimizing the chances of bias.

SAMPLE SIZE CALCULATIONS

With an assumed sensitivity / specificity of predicting lupus nephritis using urinary TWEAK as 70% and a precision of 10% and a desired confidence interval at 95% we arrived at a sample size of 81 children in each group (SLE/ SLE with lupus nephritis/ disease control/healthy controls.

Sensitivity of the new test (%) = 70(expected sensitivity /specificity of urinary TWEAK in predicting lupus nephritis

Precision (%) = 10

Desired confidence level (%) = 95

No. of diseased subjects needed = 81

FUNDING AND APPROVAL

Institutional Research Board approval

The research proposal was discussed by the Institutional Review Board in July 2014 and approval was obtained [IRB Min. No.9084 dated 06.10.2014].

There were no ethical issues related to this study**.** Institutional review board approval was obtained prior to the commencement of the study. The funds were used for the procurement of the TWEAK ELISA kits and for processing the samples

DATA COLLECTION

All children who fulfilled the inclusion criteria were enrolled into the study after explaining the study and obtaining the consent and assent forms. The data collection was done by the principal investigator in the case report form **(Annexure III)**

The following details were recorded specifically:

1) Demographics – age, sex, body mass index
2) Duration of disease
3) Disease activity scores – SLEDAI , renal SLEDAI
4) Laboratory parameters including haematological and biochemical tests.

   Assessment of disease activity using SLEDAI (SLE Disease Activity Score) was done for children in SLE group. Similarly, in children with Lupus nephritis Renal SLEDAI score was used to assess renal disease activity.

   Urine samples from all the children were collected and stored in the refrigerator. Urinary TWEAK levels were measured by using commercial ELISA kits. Urinary TWEAK levels were correlated with other markers of disease activity (anti dsDNA, C3 and C4, and renal SLEDAI score). TWEAK levels were also correlated with levels of proteinuria and class of lupus nephritis. TWEAK levels in normal healthy children were done to know the baseline normal values and served as controls.

HUMAN TWEAK ELISA KIT

This TWEAK ELISA is a quantitative competitive immunoassay which is manufactured by Neoscientific Company. Details of the assay procedure has been given in

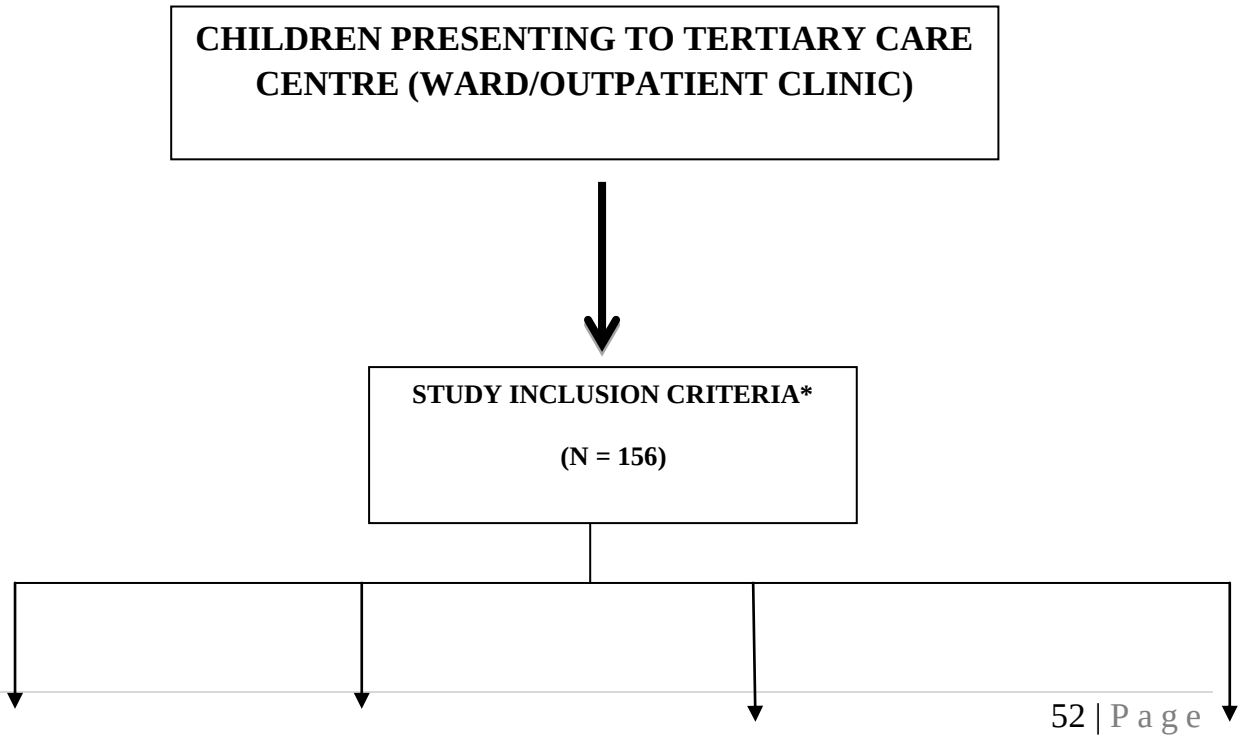
Annexure IV.

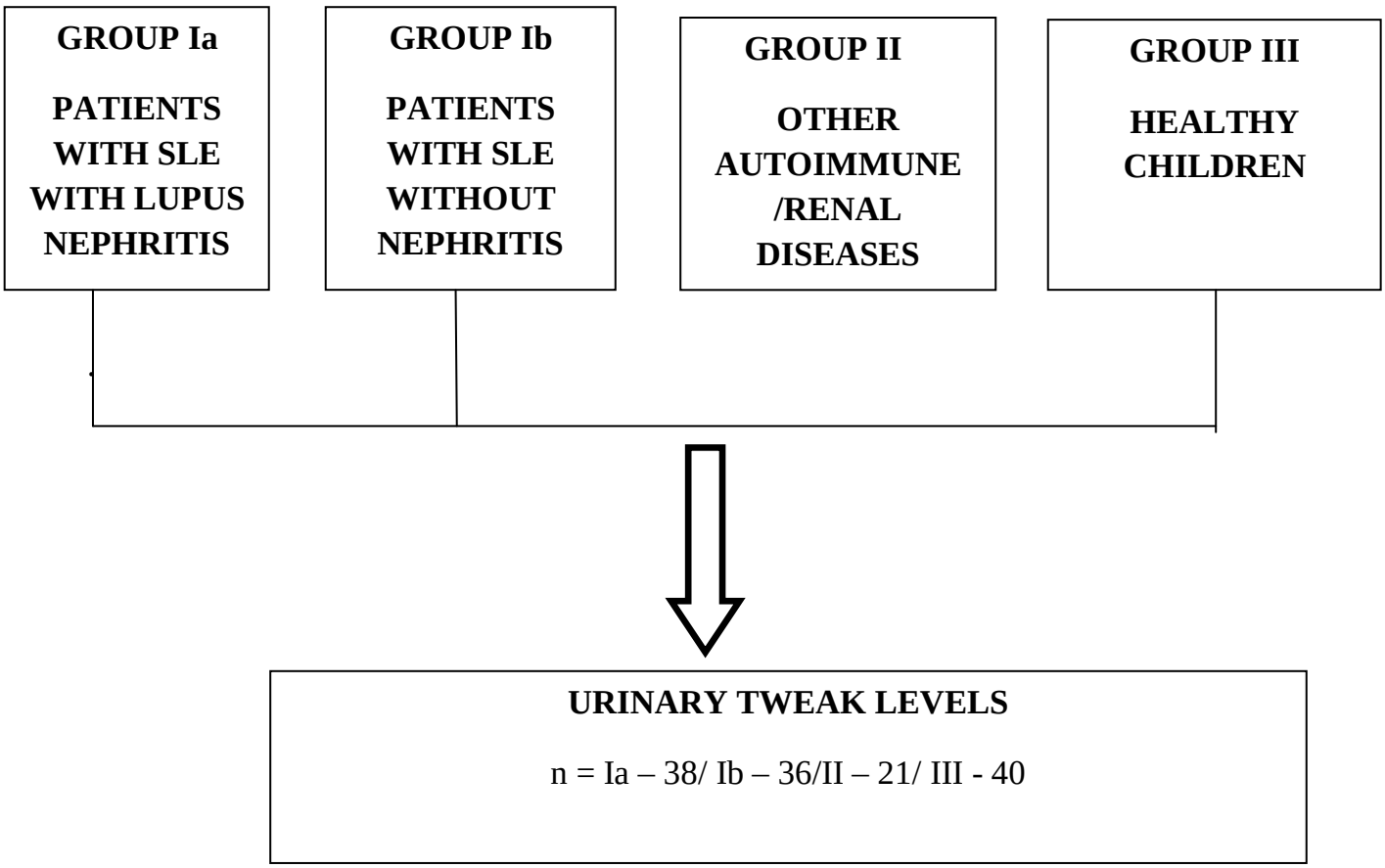
DATA ANALYSIS AND STATISTICAL METHODS

Data entry was done by the principal investigator in epidata software. (**Annexure V)** The results were analysed using STATA version 13.1 software. The statistical tests used were student t test, chi square test or Mann Withney U test, Fisher's exact test based on the normality of distribution of the variables.

Sensitivity and specificity values were calculated by using a 2x2 analysis for the diagnostic test. The validity and predictive value statistics were presented with 95 per cent confidence interval. The best cut off level of urinary TWEAK was identified using ROC analysis.

STROBE FIGURE





RESULTS

The prospective study was conducted over a period of 11 months (October 2014 – September 2015) in the Department of Child Health, Christian Medical College, Vellore, a tertiary care centre in South India. Children presenting to the outpatient department and inpatient wards who fulfilled the inclusion criteria and had none of the exclusion criteria were enrolled into the study.

Clinical history and detailed physical examination was done at the time of recruitment. The clinical data, as well as the serological and laboratory parameters (haematological and biochemical) were noted in a standardised proforma.

A total of 156 children fulfilled the inclusion criteria (40 in lupus nephritis, 36 in SLE, 40 in disease control and 40 in the healthy children group). These were included as the Study group.

All participants and their legal guardians provided written consent to participate in the study prior to recruitment.

The results were analysed in three parts :

Part I – Demographic profile

Part II – Comparison between lupus nephritis (Group Ia) and SLE without nephritis (Group Ib)

Part III – Urinary TWEAK levels comparison between lupus nephritis (Group Ia), SLE without nephritis (Group Ib) and healthy control (Group III)

PART – I: DEMOGRAPHIC PROFILE

1) Age distribution

Table 1 : AGE DISTRIBUTION

AGE	NUMBER (n = 156)	PERCENTAGE
More than 10 years	**102/156**	**65%**
Less than10 years	**54/156**	**35%**

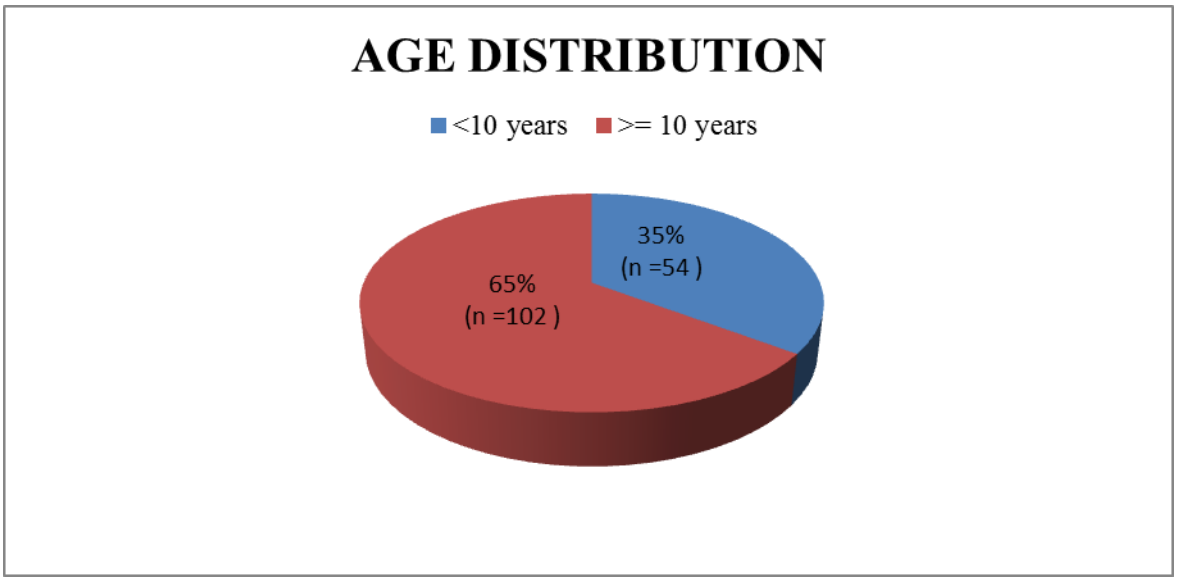


Figure 1: AGE DISTRIBUTION

Of the children recruited, 65% (102 /156) were > 10 years of age while 35% (54/156) were < 10 years of age

Table 2 : AGE DISTRIBUTION – MEDIAN AGE IN DIFFERENT GROUPS

GROUP	MEDIAN (years)	RANGE (Minimum –Maximum)
Lupus nephritis (n = 40)	**14**	**12 – 15.5**
SLE without nephritis (n = 36)	**14**	**12 - 16**
Other autoimmune/ renal disease (n = 40)	**12.5**	**6.5 - 15**
Healthy controls (n = 40)	**8**	**6.5 - 11**

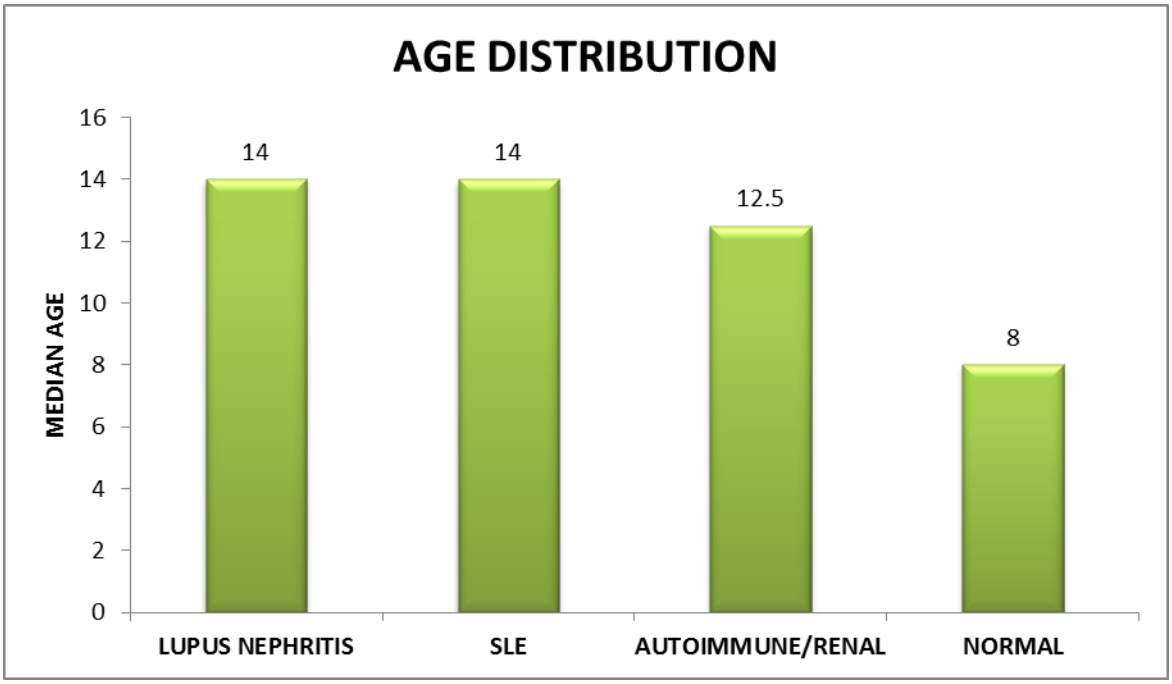


Figure 2: MEDIAN AGE DISTRIBUTION

The median age for group Ia and group Ib was 14 whereas it was 12.5 in the disease control group (other autoimmune and renal disease) and 8 in healthy controls.

2) Sex distribution

Table 3 : SEX DISTRIBUTION

SEX	NUMBER OF PATIENTS (n= 156)	PERCENTAGE
MALE	74/156	47%
FEMALE	82/156	53%

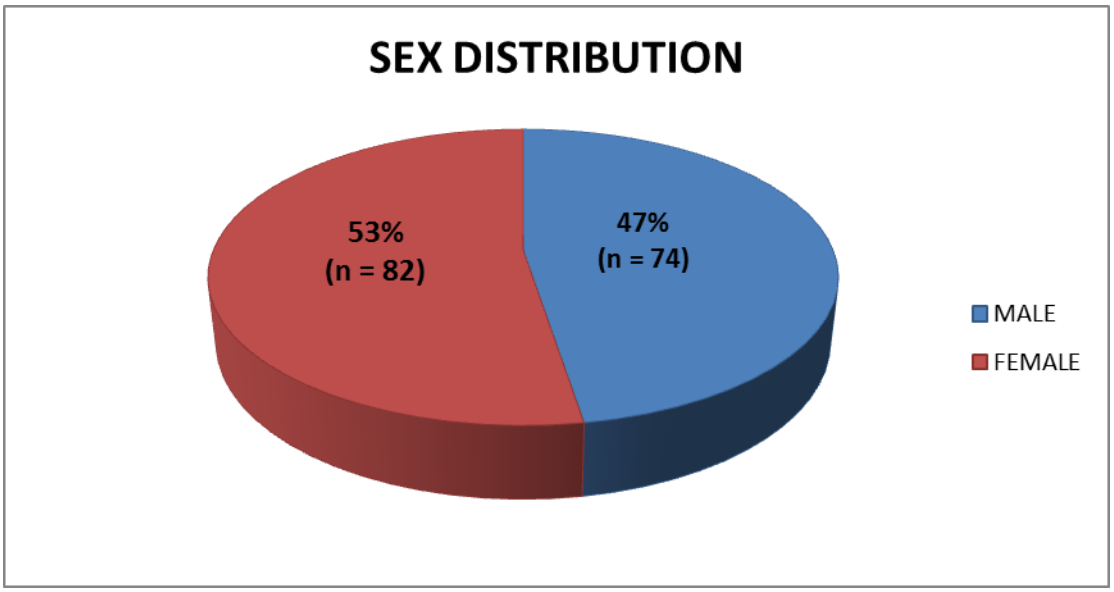


Figure 3 : SEX DISTRIBUTION

In the study group, 53% (82/156) were females whereas 47% (74/156) were males.

Table 4 : SEX DISTRIBUTION - Group specific

GROUP	MALE (Percentage)	FEMALE (Percentage)	TOTAL (n)	p value
Lupus nephritis	9 (22.5%)	31(77.5%)	40	<0.001
SLE without nephritis	10 (27.8%)	26 (72.2%)	36	
Autoimmune/ renal disease	28 (70%)	12 (30%)	40	
Healthy controls	27 (67.5%)	13 (32.5%)	40	

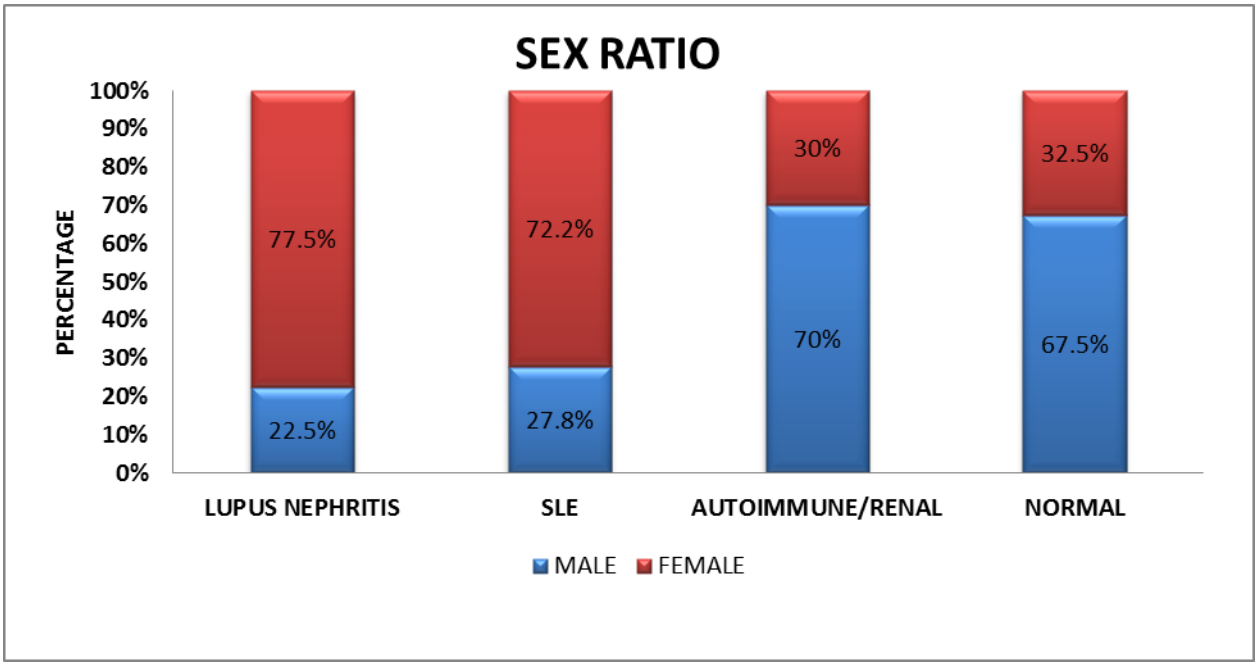


Figure 4: SEX RATIO

Both Group Ia and Ib subgroups had significant female preponderance with M:F ratio of 1 : 3.4 in Group Ia and 1 : 2.6 ratio in Group Ib ($p < 0.05$)

3) Body mass index

Table 5 : MEDIAN BODY MASS INDEX IN DIFFERENT GROUPS

GROUP	MEDIAN	RANGE (Min –Max)	p value
Lupus nephritis (n = 40)	**19**	**16.2 – 21.6**	**< 0.001**
SLE without nephritis (n = 36)	**18.4**	**15.9 – 21.2**	
Other autoimmune/ renal disease (n = 40)	**15.5**	**14.1 – 17.6**	
Healthy controls (n = 40)	**14.2**	**12.9 – 16.8**	

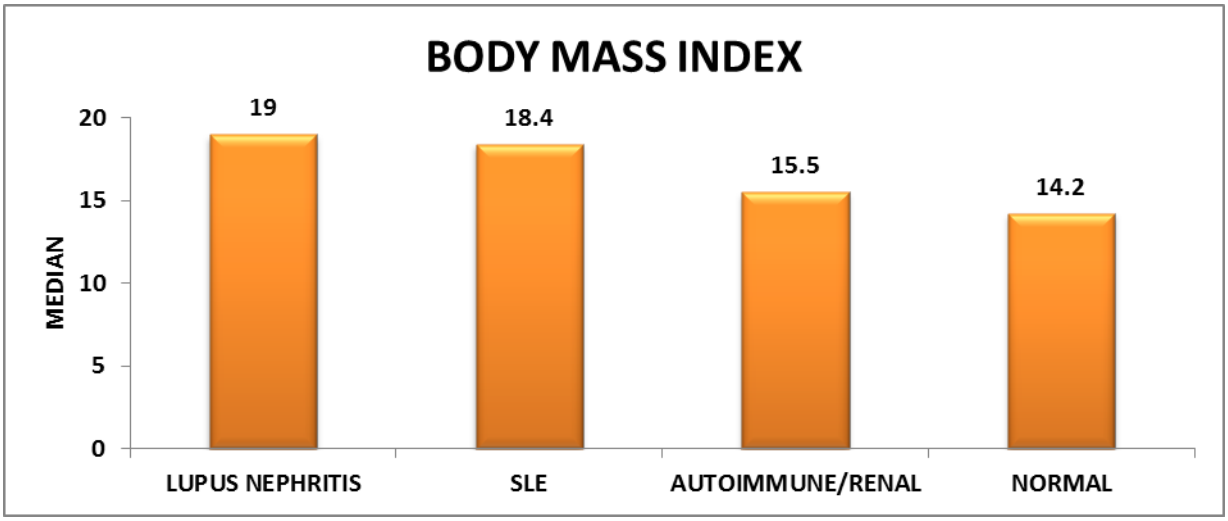


Figure 5: BODY MASS INDEX

The group Ia and Ib subgroups had body mass index of 19 and 18.4 respectively.

The disease control and healthy control had significantly lower body mass index of 15.5 and 14.2 respectively (p= <0.001)

PART II: COMPARISON BETWEEN LUPUS NEPHRITIS AND SLE WITHOUT NEPHRITIS

There were seventy six children with SLE. Of these, forty children had renal involvement (Lupus nephritis) and thirty six did not have any renal involvement (SLE without nephritis). These two groups were further compared for demography, clinical presentations and laboratory parameters.

4) Disease duration

Table 6: MEDIAN DISEASE DURATION IN MONTHS

GROUP	MEDIAN (Duration in months)	RANGE (Min –Max)	P value

Lupus nephritis (n = 40)	**24**	**7.5 - 36**	**<0.254**
SLE without nephritis (n = 36)	**17**	**6 – 27**	

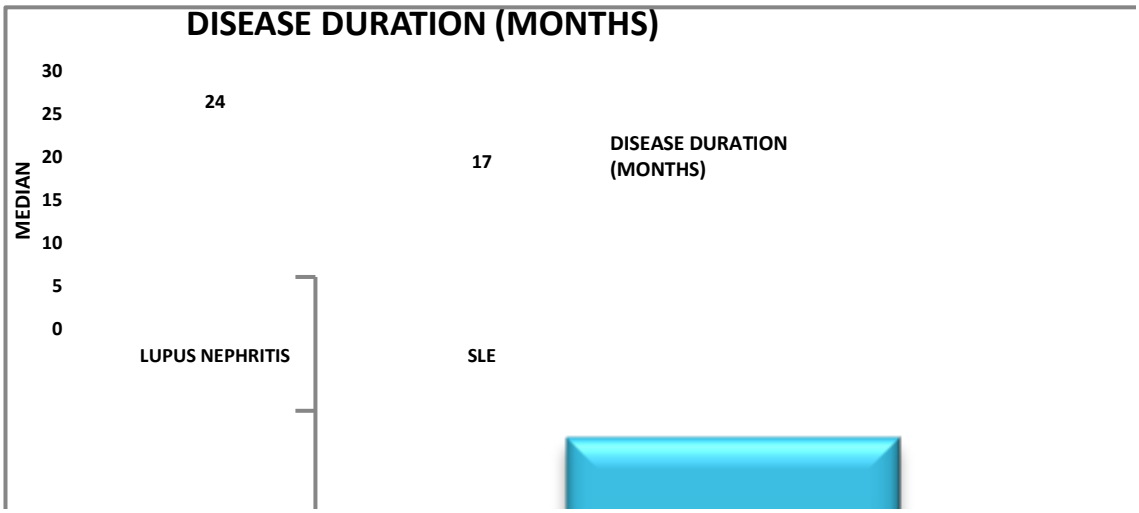


Figure 6 : DISEASE DURATION (Months)

Median duration of disease in group Ia patients was higher than that in group Ib patients

(24onths versus 17 months). The difference was not statistically significant.

5) Clinical features

Table 7 : CLINICAL FEATURES

(1997 ACR CLASSIFICATION CRITERIA)

FEATURE	LUPUS NEPHRITIS (n = 40) Number (%)	SLE without nephritis (n = 36) Number (%)	p value
ANA positive	**34 (85%)**	**36 (100%)**	**0.026**
Immune disorder	35 (87.5%)	32 (88.9%)	0.852

Arthritis	28 (70%)	23(63.9%)	0.571
Haematological	23 (57.5%)	18 (50%)	0.512
Malar rash	16 (40%)	19 (52.8%)	0.264
Oral ulcer	16 (40%)	13 (36.1%)	0.727
Photosensitivity	5 (12.5%)	11 (30.6%)	0.054
Neurological	**1 (2.5%)**	**6 (16.7%)**	**0.033**
Serositis	4 (10%)	2 (5.6%)	0.473
Discoid rash	1 (2.5%)	0	>0.99

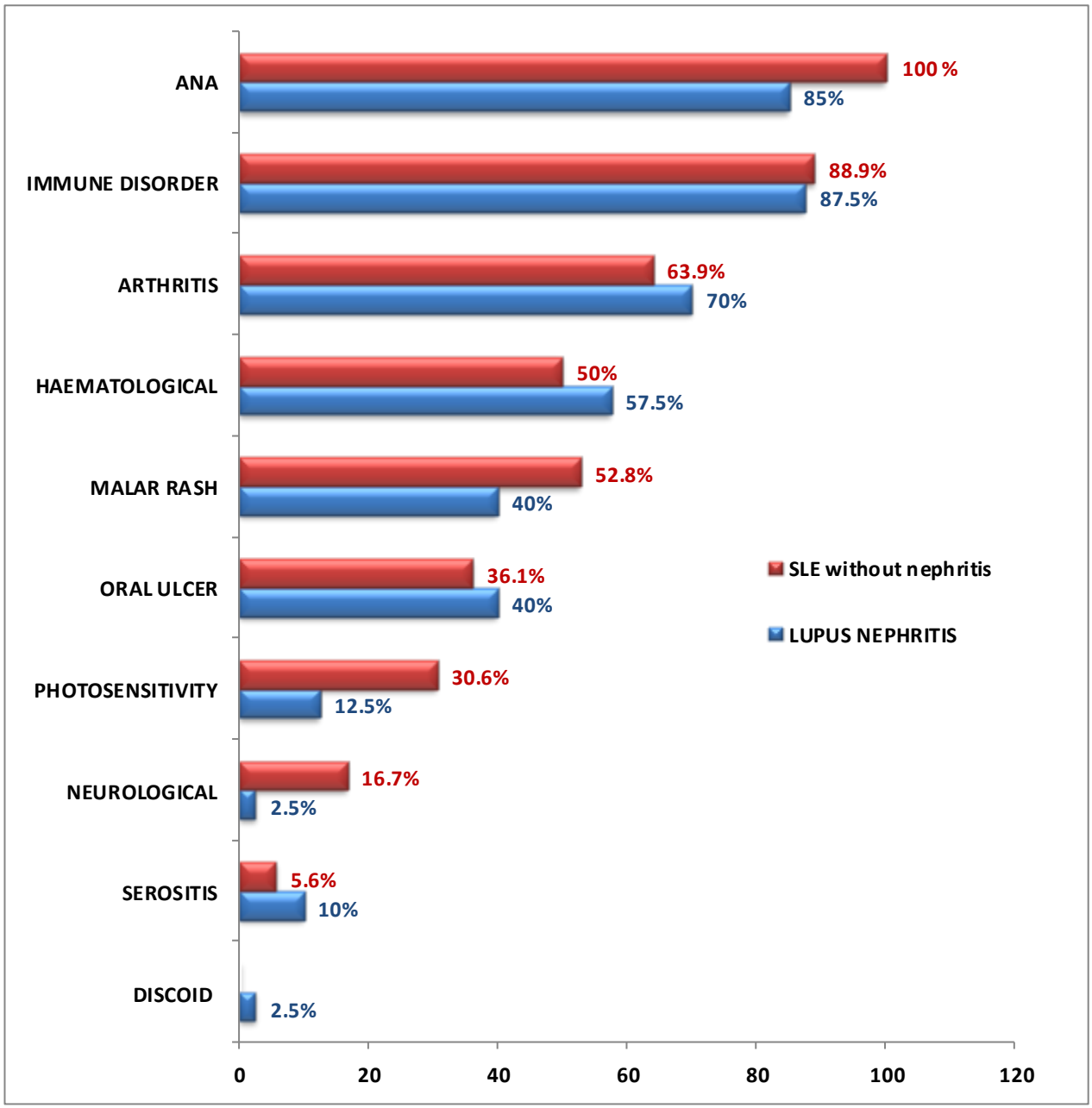


Figure 7 : CLINICAL FEATURES (1997 ACR Classification)

ANA positivity and neurological involvement was significantly more in the SLE group compared to the Lupus Nephritis group ($p<0.05$). Rest of the clinical features were similar between the two groups.

6) Renal parameters in lupus nephritis

Table 8 : RENAL PARAMETERS IN LUPUS NEPHRITIS

PARAMETERS	LUPUS NEPHRITIS (n = 40) NUMBER	PERCENTAGE (%)
PROTEINURIA **Nephrotic range**	29/40 13/29	72.5% 44.8%
HAEMATURIA	24/40	60%
PYURIA	24/40	60%
URINARY CAST	6/40	15%

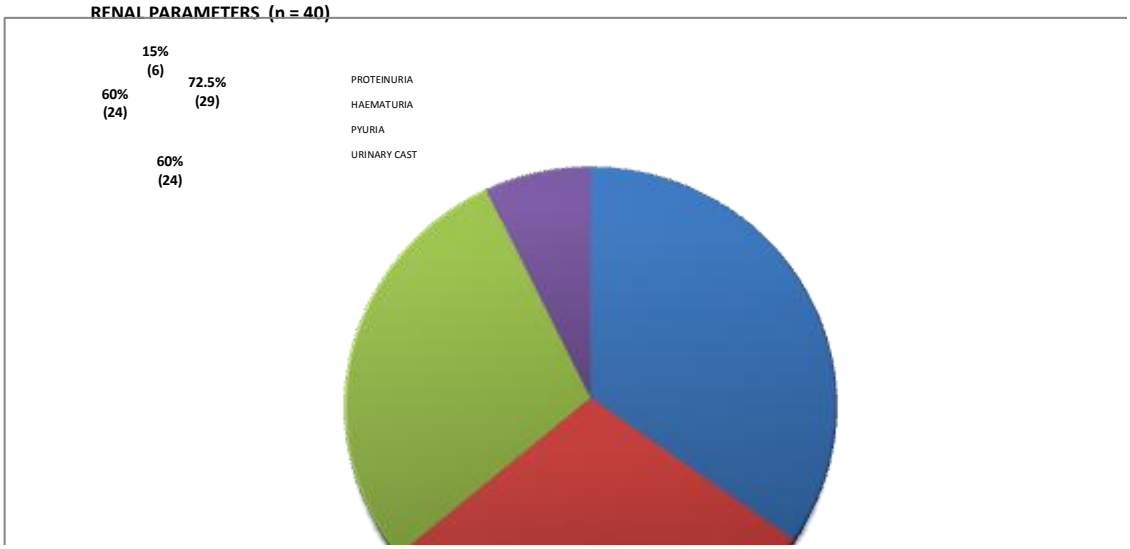


Figure 8: RENAL PARAMETERS IN LUPUS NEPHRITIS

Among those with Lupus nephritis (Group Ia), proteinuria was present in 72.5% (29/40) 60% (24/40) each had haematuria and pyuria. Only 6 patients 15% (6/40) had urinary casts.

7) Renal SLEDAI score

Table 9 : RENAL SLEDAI SCORE DISTRIBUTION

Renal SLEDAI score	NUMBER (n= 39)	PERCENTAGE (%)
ZERO	6	15%
FOUR	8	20%
EIGHT	7	17.5%
TWELVE	13	32.5%
SIXTEEN	5	12.5%

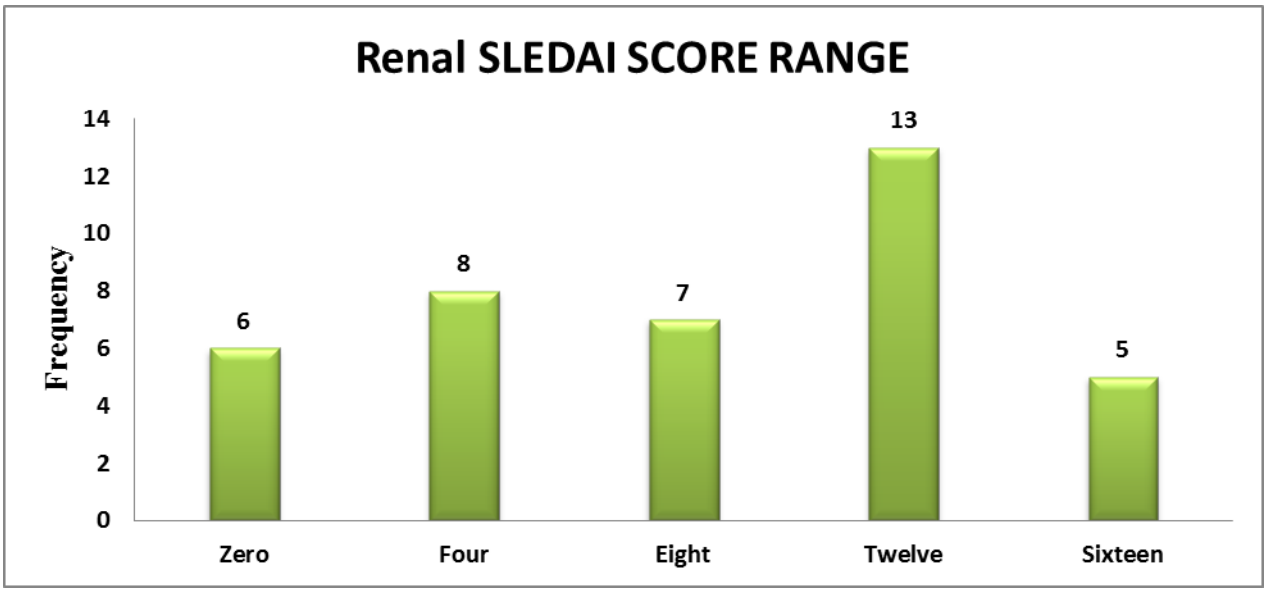


Figure 9 - RENAL SLEDAI SCORE

13/39 patients (32.5%) had renal SLEDAI score of twelve followed by 8/39 patients (20%) with renal SLEDAI score of four.

8) Classification of lupus nephritis

Table 10 : LUPUS NEPHRITIS CLASSSIFICATION AT RENAL BIOPSY

CLASS OF LUPUS NEPHRITIS	NUMBER OF PATIENTS (n = 36)	PERCENTAGE (%)
Class II (Mesangial)	3	7.5
Class III (Focal)	5	12.5
Class IV (Diffuse)	26	65
Class V (Membranous)	1	2.5
Class VI (Sclerosis)	1	2.5

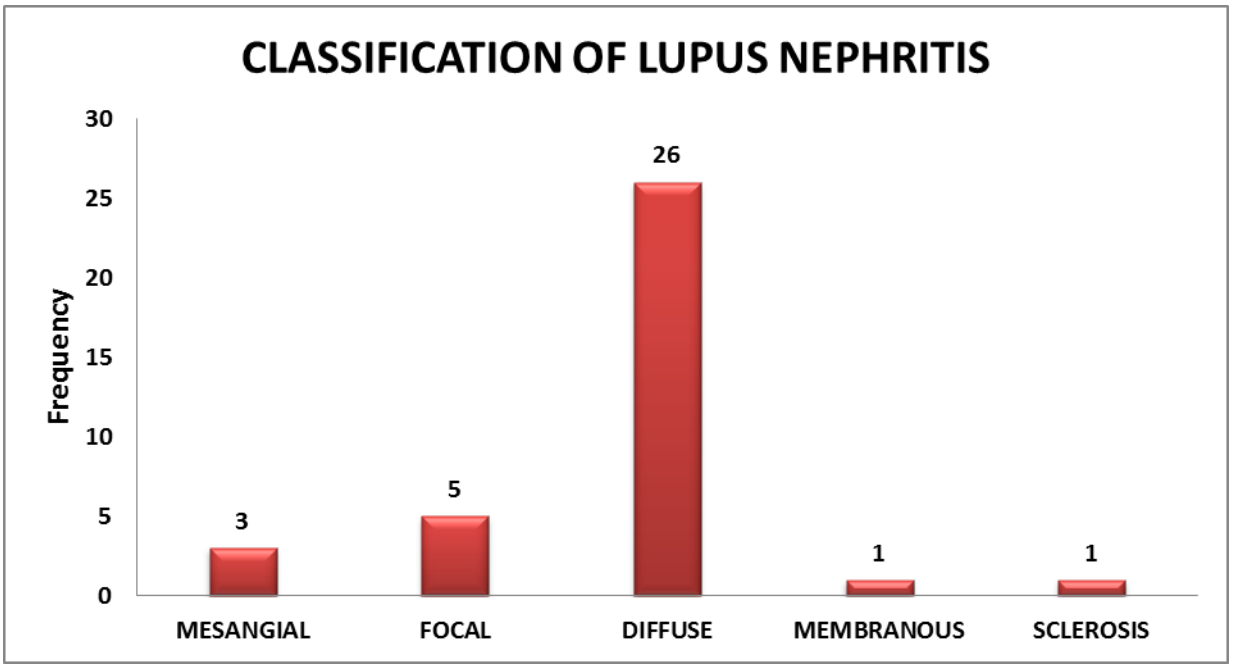


Figure 10: CLASSIFICATION OF LUPUS NEPHRITIS

The predominant renal lesion was Diffuse Proliferative GN (Class IV LN) in 65% (26/36) followed by Focal proliferative GN (Class III LN) in 12.5% (5/36)

Renal biopsy was not done for 4 patients.

9) Laboratory parameters with histopathological classification

Table 11: CORRELATION OF LABORATORY PARAMETERS WITH HISTOPATHOLOGY CLASS

PARAMETER	CLASS II (n=3)	CLASS III (n = 5)	CLASS IV (n = 26)	CLASS V (n = 1)	CLASS VI (n = 1)	p-value
PROTEINURIA (n=25)	2/25 (8%)	4/25 (16%)	**17/25 (68%)**	1/25 (4%)	1/25 (4%)	0.898
NEPHROTIC RANGE (n=13)	2/13 (15.4%)	2/13 (15.4%)	**8/13 (61.5%)**	0/13 (0%)	1/13 (7.7%)	0.492
HAEMATURIA (n=21)	2/21 (9.5%)	3/21 (14.3%)	**15/21 (71.4%)**	0/21 (0%)	1/21 (4.8%)	0.942
PYURIA (n=19)	2/19 (10.5%)	4/19 (21%)	**13/19 (68.4%)**	0/19 (0%)	0/19 (0%)	0.514
URINARY CASTS (n=6)	1/6 (16.7%)	1/6 (16.7%)	**3/6 (50%)**	0/6 (0%)	1/6 (16.7%)	0.178
HTN (n=6)	2/6 (28.6%)	0/6 (0%)	**5/6 (71.4%)**	0/6 (0%)	0/6 (0%)	0.345

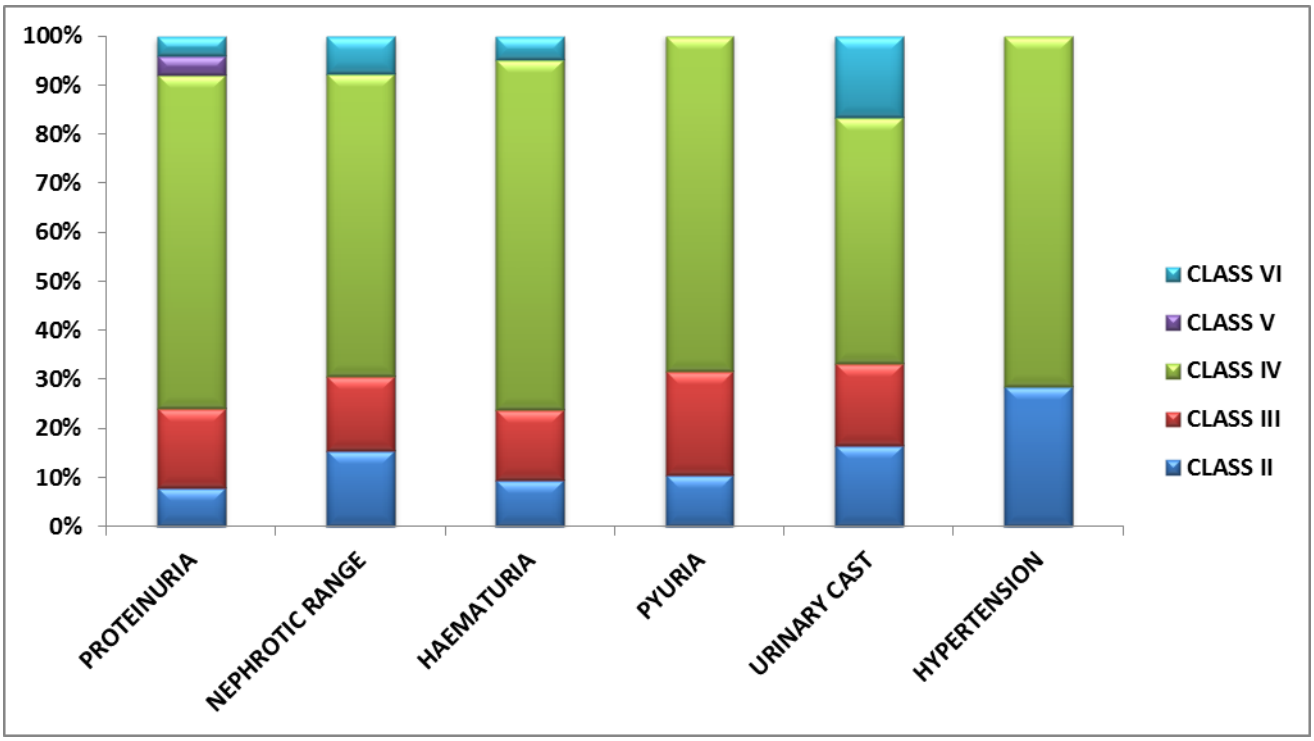


Figure 11: Comparison of laboratory parameters with classification of lupus nephritis

Class IV lupus nephritis group was reported in 61.5% with nephrotic range proteinuria, 71.4% in those with haematuria, 71.4% in those with hypertension, 68.4 % in those with pyuria and 50% in those with urinary casts.

None of these were statistically significant.

10) Clinical characteristics of Lupus nephritis group

Table 12 : PARAMETERS AMONG LUPUS NEPHRITIS GROUP

PARAMETERS	NEWLY DIAGNOSED (n = 13)	RELAPSE (n = 21)	REMISSION (n = 6)	p value
Ds DNA	492 ± 307.6	379.2 ± 385.64	32.67 ± 32.44	0.14
Urine protein creatinine ratio	2.62 ± 2.47	3.11 ± 3.11	0.07± 0.04	0.06
C3	**44.2 ± 31.1**	**65.14 ± 36.78**	**98.07 ± 24.31**	**0.009**
C4	**9.0 ± 4.9**	**13.85 ± 9.28**	**19.38 ± 9.01**	**0.039**
Creatinine	0.7 ± 0.2	0.74 ± 0.43	0.57 ± 0.02	0.556
Blood pressure **Systolic BP** **Diastolic BP**	 114.8 ± 23.3 73.2 ± 15.3	 120.29 ± 16.98 74.67 ± 17.17	 102.5 ± 10.73 67.83 ± 16.59	 0.131 0.674
Treatment (%) **Prednisolone** **Mycophenolate mofetil**	 84.6% **15.4%**	 76.2% **57.1%**	 50% **66.7%**	 0.265 **0.03**

C3 and C4 levels were significantly lower in the newly diagnosed group compared with those on treatment (with relapse or remission) (p value < 0.05).

Differences of other comparisons in these groups of laboratory parameters and blood pressure were not statistically significant.

Most newly diagnosed patients were on prednisolone. Only 2 newly diagnosed lupus nephritis patients and a significant number of old patients were on Mycophenolate mofetil.

11) Comparison of laboratory parameters

Table 13 : LABORATORY PARAMETERS BETWEEN LUPUS NEPHRITIS AND SLE WITHOUT NEPHRITIS

VARIABLES	LUPUS NEPHRITIS (n = 40) MEAN ± SD	SLE WITHOUT NEPHRITIS (n = 36) MEAN ± SD	MEAN DIFFERENCE	p VALUE
Anti ds DNA	363.5± 357	245.3 ± 232.3	118.2	0.33[a]
C3	**63.2 ± 13.1**	**82.6 ± 5.5**	**- 19.4**	**0.02**
C4	13.1 ± 8.6	11.3 ± 5.3	1.8	0.3
Urine protein/ Creatinine ratio	**2.5 ± 2.8**	**0.2 ± 0.3**	**2.3**	**<0.001**
Creatinine	**0.7 ± 0.3**	**0.5 ± 0.08**	**0.2**	**0.002[a]**
Hypertension	**SBP = 115.8 ± 19.2** **DBP = 73.2 ± 16.2**	**SBP = 104.7 ± 10.2** **DBP = 64.1 ± 10.1**	**11.1** **9.1**	**0.003** **0.005**

[a] Mann-Whitney U test was used to assess the significant difference

C3 levels, urine protein creatinine ratio and serum creatinine levels showed significant difference between Lupus Nephritis and SLE patients without Nephritis (p= < 0.05)

Systolic and diastolic blood pressure was found to be significantly higher among Group 1a Lupus Nephritis patients (p value < 0.05)

12) Medications

Table 14 : MEDICATION PROFILE

MEDICATIONS	LUPUS NEPHRITIS n= 40 (%)	SLE WITHOUT NEPHRITIS n = 36 (%)
PREDNISOLONE	30/40 (75%)	20/36(55.5%)
HYDROXYCHLOROQUINE	32 /40(80%)	28/36 (77.7%)
MYCOPHENOLATE MOFETIL	18/40 (45%)	2 /36(5.5%)
AZATHIOPRINE	8/40 (20%)	3/36 (8.3%)
CYCLOPHOSPHAMIDE	4/40 (10%)	0
OTHERS	3/40 (7.5%)	0

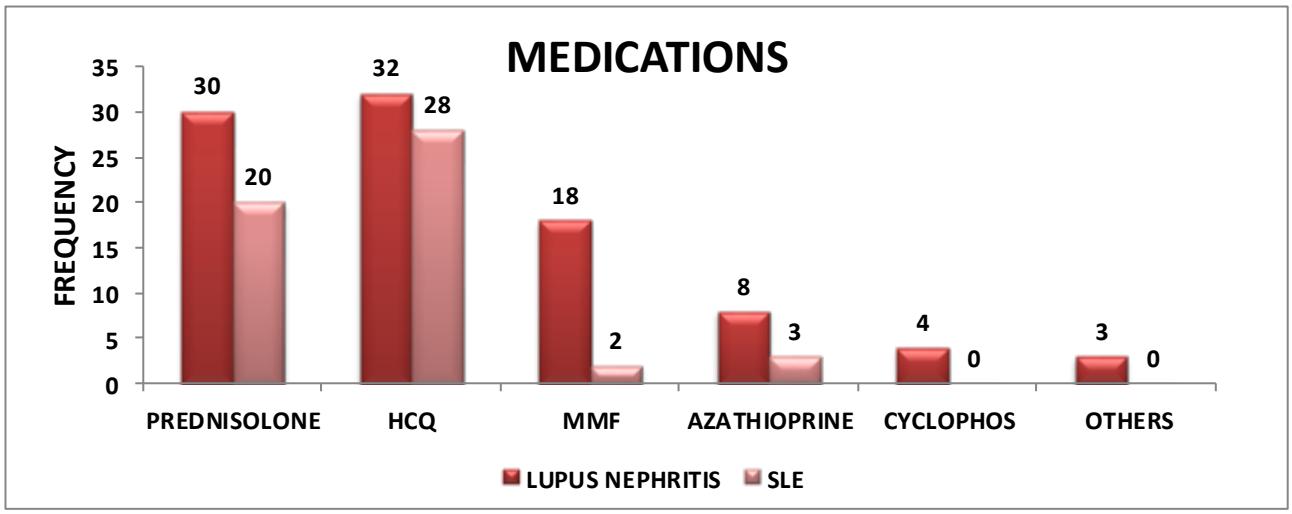


Figure 12: MEDICATIONS

A total of 75% (30 /40) of the patients with lupus nephritis were on corticosteroids as compared to 55.5% of SLE patients (20/36).

The most commonly used second line drug in both groups was hydroxychloroquine. Mycophenolate mofetil were more commonly used among Lupus nephritis as compared to SLE patients (45% versus 5.5%).

PART III: URINARY TWEAK LEVELS BETWEEN GROUPS IN THE COHORT

URINARY TWEAK BETWEEN LUPUS NEPHRITIS, SLE without nephritis AND HEALTHY CONTROLS

Urinary TWEAK levels were done among the four groups – Group Ia, Ib, disease controls and healthy controls. The results were analysed and compared among the four groups and also with other laboratory parameters. Urinary TWEAK level was also correlated with the classification of lupus nephritis as well as renal disease activity which is described by renal SLEDAI score of ≥ 4.

Table 15 : Urinary TWEAK level

Variable	Median	Mean± STD	Range
Urinary TWEAK	3.32	3.49 ± 2.29	0.02– 10.35

The mean level of Urinary Tweak levels for the cohort was 3.49 ± 2.29 ng/ml, ranging from 0.02 to 10.35 ng/ml.

13) Urinary TWEAK levels between study groups

The box plot of the comparison is shown in Figure 13.Statistical tests to ascertain significance was done between groups using one-way ANOVA.

Table 16 : Urinary TWEAK between different groups

GROUP	URINARY TWEAK LEVEL(MEDIAN)	MEAN ± SD	p VALUE
LUPUS NEPHRITIS(Gp 1a)	3.17	3.26 ± 2.55	0.888
SLE WITHOUT LUPUS NEPHRITIS(Gp 1b)	3.47	3.47 ± 2.18	
AUTOIMMUNE/ RENAL (Gp II)	3.42	3.67 ± 2.44	
HEALTHY CONTROLS(Gp III)	3.03	3.63 ± 2.12	

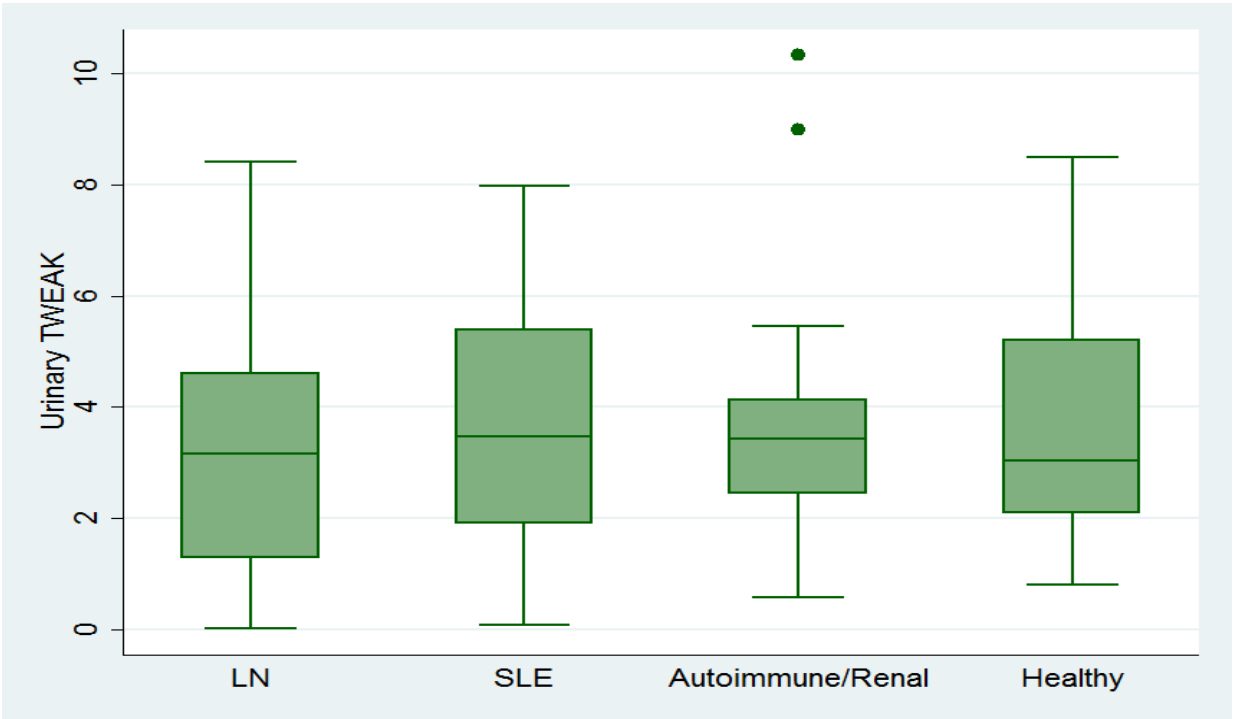


Figure 13: Comparison of urinary TWEAK between different groups

The Urinary TWEAK levels were highest in the SLE without Nephritis group, closely followed by the Autoimmune /Renal disease group. The SLE with nephritis group had lower values, but was higher than the healthy controls. However, the difference between these groups was not statistically significant. (p value = 0.888).

14) Comparison between renal SLEDAI score and urinary TWEAK levels

One-way ANOVA was used for assessing the significant difference between the renal SLEDAI scores.

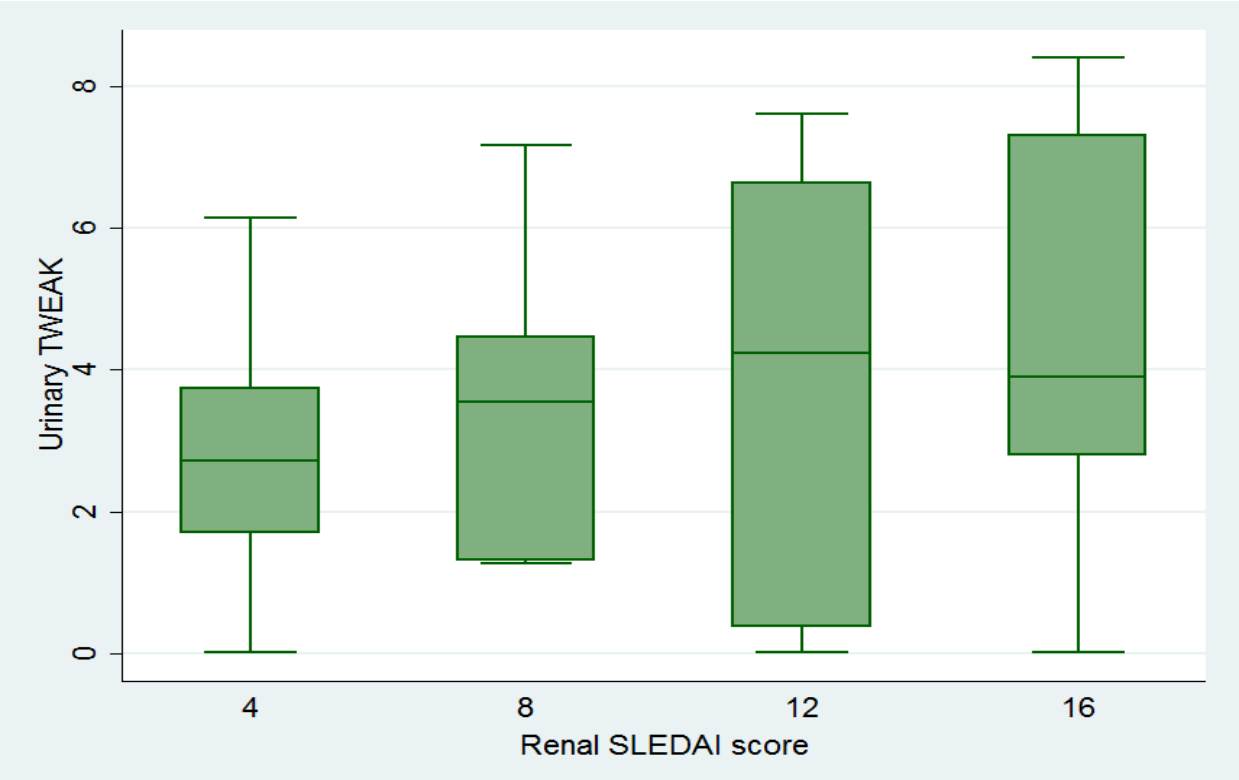


Figure 14: Comparison between renal SLEDAI and urinary TWEAK levels

Higher renal SLEDAI score had a higher median value of urinary TWEAK. However the difference was not statistically significant (p = 0.743).

15) TWEAK levels and class of lupus nephritis

Figure 15: Comparison of urinary TWEAK levels between lupus nephritis classes

Maximum levels of TWEAK were seen in Class III (focal) followed by class II (mesangial) and class IV (diffuse) lupus nephritis.

This difference was not statistically significant (p – 0.489).

16) Urinary TWEAK levels and ds DNA antibodies

A Pearson's Correlation was run to assess the correlation between Urinary TWEAK levels and ds DNA antibodies.

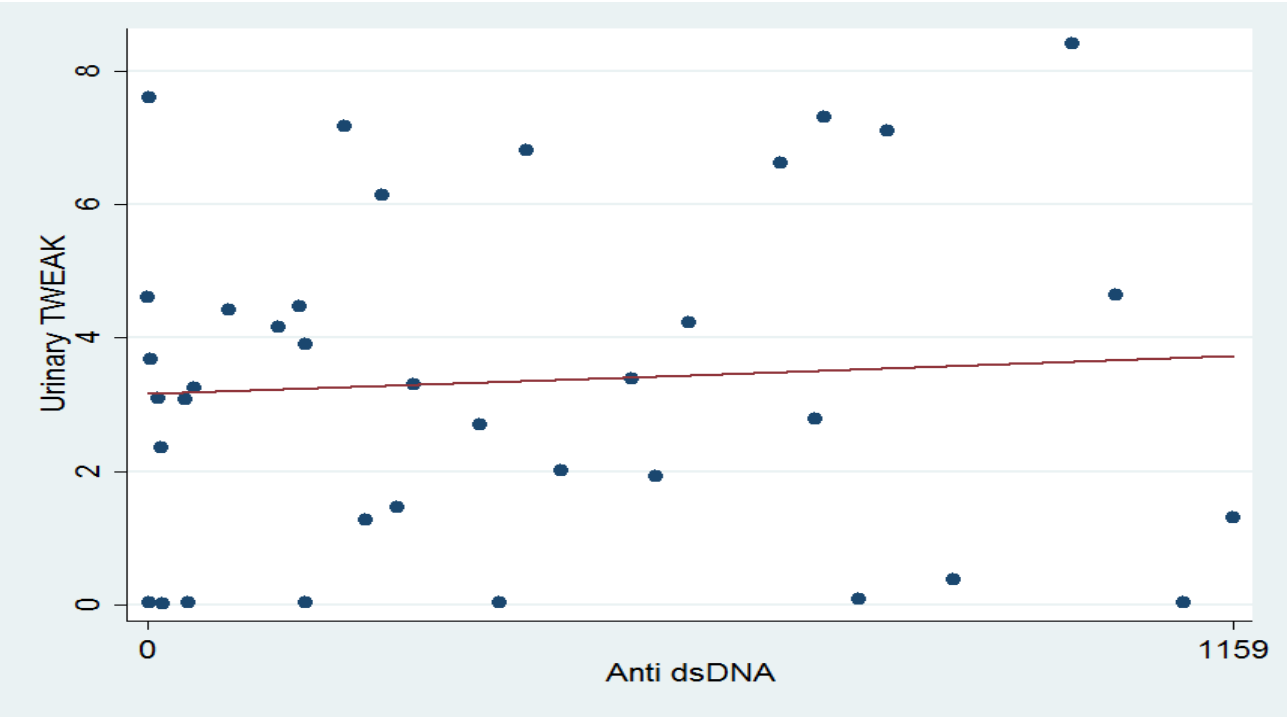


Figure 16: Comparison of urinary TWEAK levels with anti ds DNA antibodies

There was negligible positive correlation between TWEAK levels and ds DNA antibodies with r = 0.07. The correlation was not statistically significant (p = 0.686).

17) Urinary TWEAK levels and serum creatinine

Pearson's correlation was used to assess correlation between urinary TWEAK and serum creatinine levels.

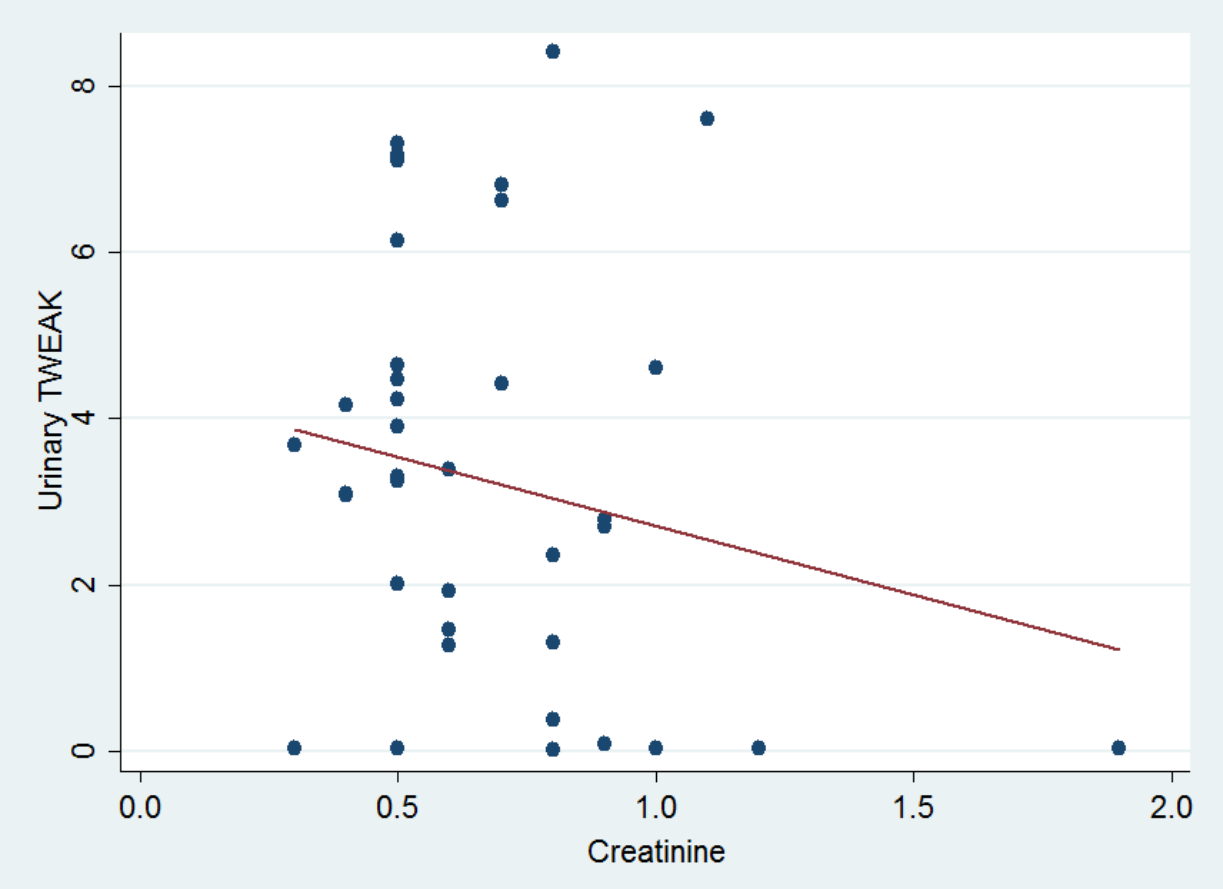


Figure 17: Comparison of urinary TWEAK levels and serum creatinine

There was a small negative correlation between the serum creatinine levels and urinary TWEAK levels.

However this was not statistically significant (p= 0.234, r = - 0.19)

18) Urinary TWEAK levels and urine protein creatinine ratio

Pearson's correlation method was used for analysis

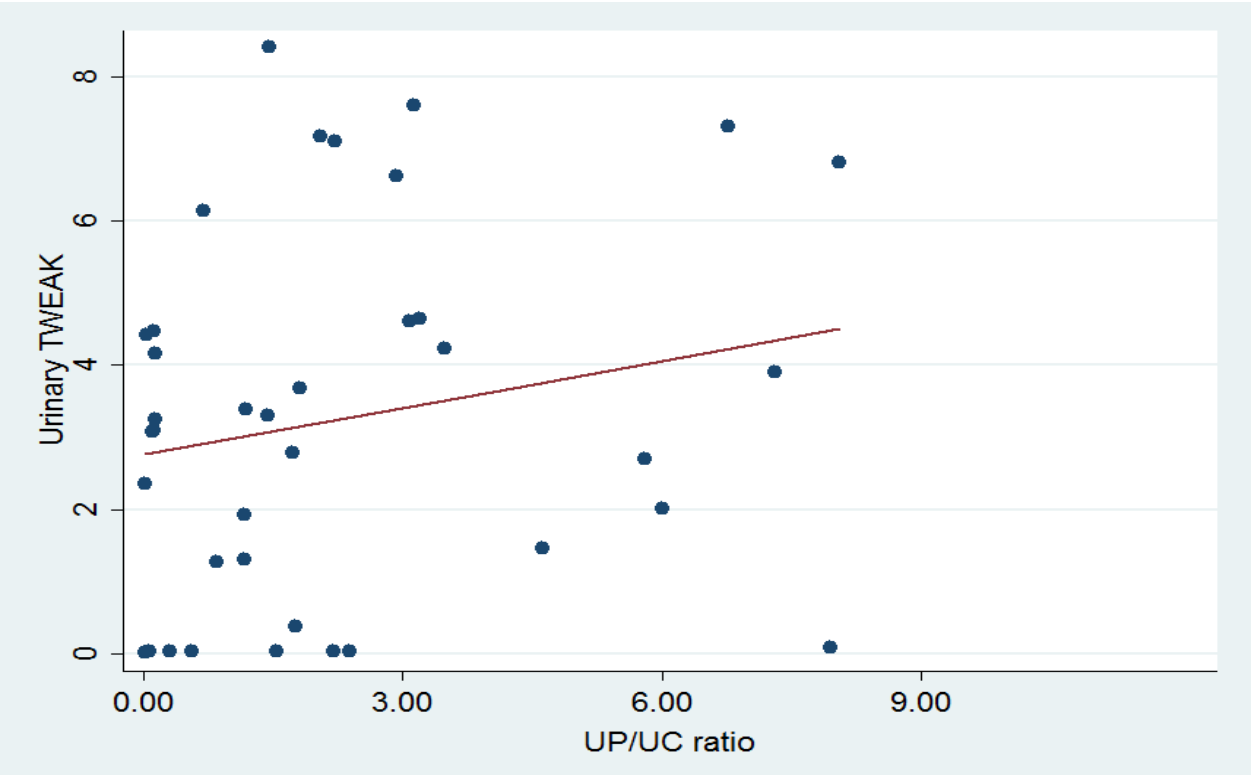


Figure 18: Comparison of urinary TWEAK levels with urine protein creatinine ratio

The correlation coefficient for urine protein creatinine ratio was 0.2, indicating a positive correlation; this was not statistically significant (p= 0.228).

19) Urinary TWEAK levels and complement C3 levels

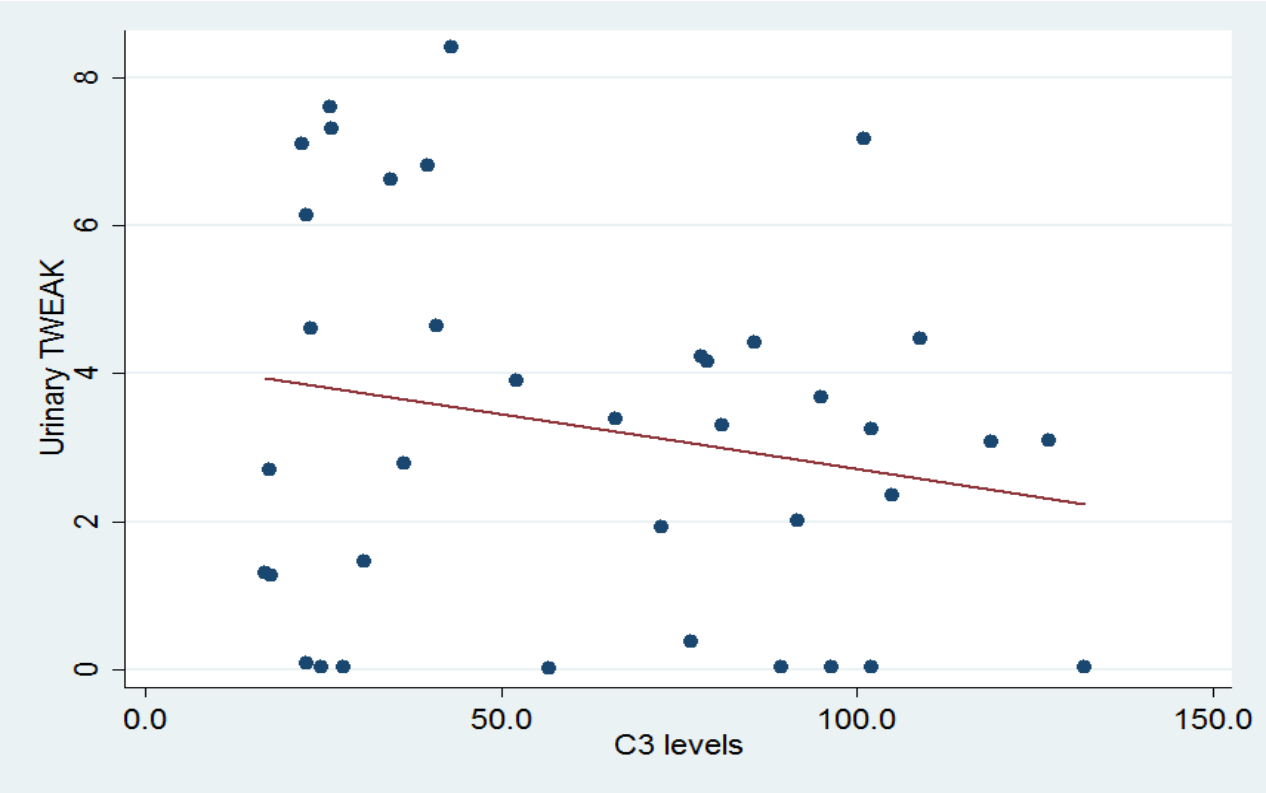


Figure 19: Comparison of urinary TWEAK levels and complement C3 level

There was a negative correlation between TWEAK levels and C3 ($r = -0.21$, $p = 0.21$).

20) Urinary TWEAK levels and complement C4 levels

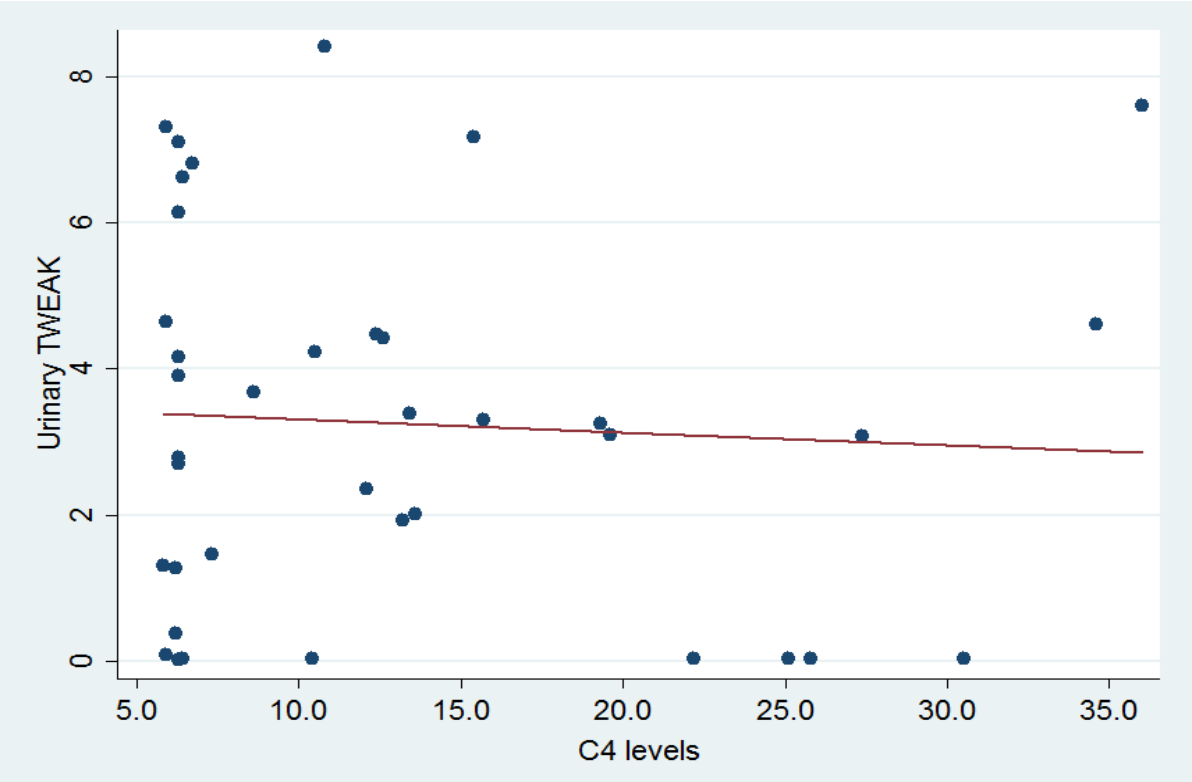


Figure 20: Comparison of urinary TWEAK levels and complement C4 levels

There was a negative correlation between TWEAK levels and C4 ($r = -0.06$, $p = 0.72$).

21) Sensitivity and Specificity of TWEAK levels

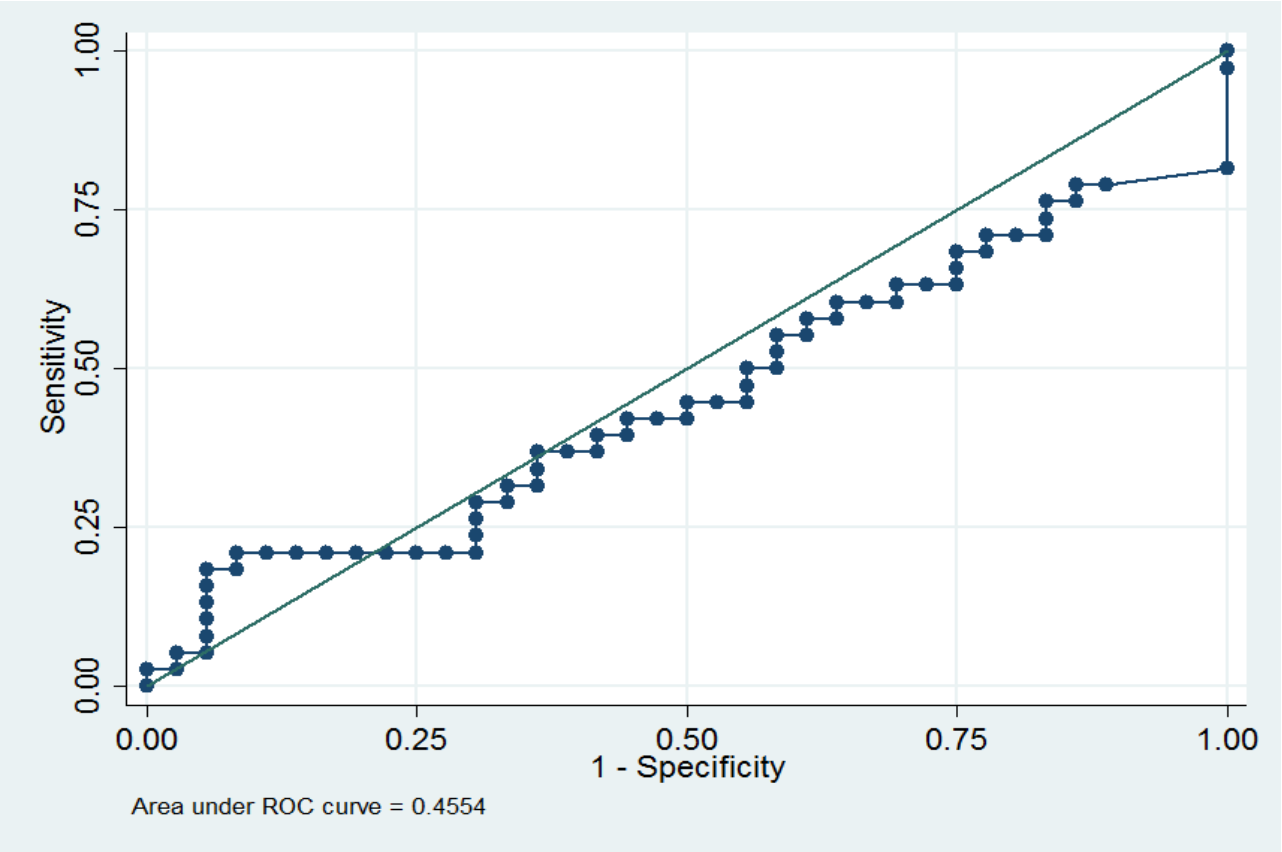


Figure 21: ROC curve for sensitivity and specificity of urinary TWEAK

ROC curve was plotted for determining the sensitivity and specificity

The area under the curve was 0.46.

The sensitivity of TWEAK for determining the disease activity was 60.53% and the specificty was 36.11%.

The cut off value for determining the active disease was reported as $\geq$ 2.7 ng/ml

DISCUSSION

This prospective study was done to assess the utility of urinary TWEAK for diagnosis of lupus nephritis and its correlation with other markers of lupus nephritis. The study was conducted in the Department of Child Health (Paediatric Nephrology and Rheumatology divisions) at Christian Medical College, Vellore.

There were 156 children enrolled in the study (40 in lupus nephritis, 36 in SLE without nephritis, 40 in other autoimmune and renal disease and 40 in healthy control groups)- all of whom fulfilled the Inclusion criteria and had none of the exclusion criteria. Demography, clinical presentations and laboratory parameters were further analysed among patients with Lupus nephritis and those SLE without nephritis.

Demographic characteristics

SLE is an autoimmune disease which predominantly affects women of child bearing age and young female adolescents. According to literature, about 15-20% of SLE cases are seen among children less than 18 years of age (1,32,33,55).

In our study, the median age of SLE patients was 14 years- both in the Lupus Nephritis group (Goup 1 a) and those without renal involvement)(Group 1b). The age distribution was slightly higher when compared to other Indian studies. In Agarwal et al study (8), the mean age group of SLE was reported to be 10.5 year while Singh et al (30)reported 10 years. Many of the patients in our study had been treated for varying duration prior to presentation and subsequently referred to our centre at a later stage of the disease. The higher mean age may be due to delayed diagnosis or due to poor

response to therapy in our institution we now see children up to 18 years of age. All these reasons may account for a higher mean age in our study population.

Timing of renal biopsy may have also resulted in a higher mean age. Initially, all children with SLE in our institution underwent renal biopsy at the time of presentation, irrespective of any clinical or laboratory indicators of renal involvement. However over the last few years the practice has changed. Currently renal biopsy is done only in patients with definite features of renal involvement as indicated by presence of proteinuria, haematuria, pyuria, urinary cast, hypertension and elevated creatinine. This change in our institution protocol may have contributed to the higher mean age group among lupus nephritis as the children are older.

SLE disease is a disease with female preponderance. In our study we also had a female: male ratio of 1:3.4 in lupus nephritis group and 1: 2.6 in SLE group. It was comparable to Western studies (35) but was lower when compared to other Asian countries. Huang JL et al, 2004 reported prevalence among girls to be 6.2 times higher than those among boys (57). A study done in our own institution by Agarwal et al, 2009 reported a male : female ratio of 1:6 (8). However Singh et al (83) reported a similar sex distribution to our study (male : female ratio of 1:4). Whether this lower female: male ratio in our study is due to the fact that lesser number of female children are being brought to medical attention is not known.

In our study, body mass index among SLE and Lupus nephritis groups were higher (19 versus 18.4) when compared to healthy children. Most of the children with SLE and lupus nephritis groups are on prolonged steroid therapy which could explain the higher

body mass index in this group of patients. Previous studies have not looked into the body mass index among this group of patients nor have they compared with normal healthy children.

In our study, the median duration of disease in lupus nephritis patients was higher than that in patients with SLE without lupus nephritis (24 months versus 17 months). Similar finding was shown by Singh et al with onset to lupus nephritis occurring 9.4 ± 12.6 months after the initial diagnosis of SLE (83). As discussed earlier, this late presentation of lupus nephritis may be due to delayed diagnosis, late referral to a tertiary centre like ours or renal biopsy being done only after the clinical evidence of renal invlovement.

Clinical manifestations

Clinical features (based on 1997 ACR classification) of SLE were compared between the Group Ia and Group Ib patients. Both the groups had similar clinical presentation. The most common feature was ANA positivity followed by serological evidence of an immune disorder. ANA was positive in all SLE patients (100%) but in only 85% of lupus nephritis patients. This was comparable to other studies like Mackie et al (35), Lee et al (36) and Singh et al (83). Immune disorder as characterized by elevated anti Ds DNA was present in 87.5% and 88.9% of patients with Group Ia and Group Ib respectively. This was similar to Indian studies (8,83) but lower when compared to other Western and Asian countries (35,36).The reason for this difference is unclear.

The other common non renal manifestations were musculoskeletal followed by haematological and mucocutaneous involvement. Musculoskeletal involvement in

Group Ia and Group Ib was 63.9% and 70% respectively. This finding was similar to Mackie et al (100) and Agarwal et al (8) but was higher compared to other Asian countries (25,36,83). The occurrence of haematological involvement was seen in 57.5% of Group Ia and 50% in Group Ib patients which was found to be lower when compared to Western study (100) but higher than those reported in other Asian countries (25,36,83). Mucocutaneous involvement like malar rash was comparable with other Indian studies (7,8,30) but occurrence of oral ulcers was higher when compared to other Asian studies (30,57). Other rarer manifestations included serositis, neurological involvement and discoid rash. These were the least common features in other studies as well (8,25,30,36,100)

Table 17 : Comparison of clinical features of SLE in world

Table 17 shows the different patterns of clinical feature distribution among SLE patients across the world

Clinical features (%)	Present study	India Agarwal et al	Singapore Tan et al	Taiwan Lee et al	Australia Mackie et al
ANA positive	100	-	98.4	98.9	100
Immune disorder	88.9	77.1	90.6	94.1	94
Arthritis	63.9	65.7	56.3	37	76
Haematological	50	60	90.6	52.9	77
Malar rash	52.8	57.1	45.3	66.7	47
Oral ulcer	36.1		32.8	34.9	17
Photosensitivity	30.6	51.5	15.6	27.5	31
Neurological	16.7	21.4	12.5	9.0	3
Serositis	5.6	2.8	7.8	13.2	14
Discoid rash	0	0	15.6	3.7	10

Renal involvement is one of the most common clinical manifestations of paediatric SLE. It is more active in childhood and is one of the major factors determining the survival among these children (2,3,10,33,59). Most often found renal involvements are nephritic range proteinuria, haematuria, hypertension and renal failure (9, 38, 39) .

The renal parameters in lupus nephritis were evaluated in our study. The most common feature was noted to be proteinuria (72.5%) followed by haematuria and pyuria (60%) and urinary casts (15%). This was in keeping with other Asian and Indian studies which showed proteinuria to be the most common feature (9,25,36,83). Although urinary sediment is supposed to correlate with severity of renal disease, in our study, presence of urinary cast was found to be low among lupus nephritis group.

Lupus nephritis is divided according to the WHO classification into 6 groups based on the histopathological changes. It is well known that the most common histological lesion is Class IV lupus nephritis.

In our study, Class IV (Diffuse segmental proliferative glomerulonephritis) lupus nephritis was the most common histopathological findings (63%) followed by Class III (Focal proliferative glomerulonephritis) lupus nephritis in 12.5% of the patients. This similar to previous Indian studies as well as other Asian studies which showed Class IV to be the commonest followed by Class II lupus nephritis (8,9,32,62,83).

Similar higher occurrence of Class IV and III lupus nephritis was found in Western studies also (12, 93). Due to various factors like late referral to tertiary centres, histological changes preceding clinical manifestations, renal biopsy being done only after the presence of renal manifestations, late diagnosis of lupus nephritis occurs and

often more severe renal damage. This warrants the need for early screening of children with SLE for renal involvement.

Table 18 : Classification of lupus nephritis in world

Classification (%)	Present study	India Agarwal et al	Singapore Tan et al	Taiwan Lee et al	Australia Mackie et al
CLASS I	0	3.7	0	1	0
CLASS II	7.5	44.4	0	11.1	0
CLASS III	12.5	4.3	23.8	11.1	18.1
CLASS IV	65	44.4	33.3	69.7	72.7
CLASS V	2.5	1.8	4.8	7.0	9.0
CLASS VI	2.5	_	_	0	_

Table 18 shows distribution of different class of lupus nephritis from various studies across the world.

Our study showed Class IV lupus nephritis group was associated with nephrotic range proteinuria (61.5%), haematuria (71.4%), hypertension (71.4%), pyuria (68.4%) and urinary casts (50%). Most studies have shown Class IV to be more commonly associated with severe renal features like proteinuria and haematuria (8,39,62,83). Agarwal et al showed 58.1% proteinuria and 66.6% haematuria in class IV lupus nephritis.

Comparison between SLE without nephritis and lupus nephritis groups

All the laboratory parameters used in the diagnosis of SLE were looked at. This included dsDNA, which is believed to correlate well with renal involvement. However comparison of anti ds DNA levels between the two groups Ia and Ib did not show any statistically significant difference. This limitation of anti ds DNA level in differentiating renal involvement in SLE patients has been described in previous literature (13–15). However both complement C3 level and urine protein creatinine ratio showed significant corelation with disease activity among Group Ia patients as compared to those in Group Ib (p value < 0.05). Higher serum creatinine was found among Group Ia when compared to Group I b patients (p value < 0.05)- this was also statistically significant. Both systolic and diastolic mean blood pressures were also significantly elevated among lupus nephritis group (p value < 0.05). Proteinuria, hypertension and elevated creatinine are the known renal parameters which are indicative of renal damage. These parameters have also been found to be present in our lupus nephritis group.

Treatment

Treatment modalities vary according to regime preference in different centres. In our centre corticosteroid was the mainstay of treatment in both Group Ia and Ib patients and is followed in many centres worldwide (8,11,35,83,101). The second most common second line drug was hydroxychloroquine as medication. Studies have shown good results in lupus nephritis following the use of Mycophenolate mofetil(11,101,102). The use of this drug in patients in our own institute has increased over the last 6 years (8)

URINARY TWEAK ANALYSIS

Urinary TWEALK was analyzed using Human TWEAK ELISA KIT. Urinary levels of TWEAK were compared between the four groups: lupus nephritis, SLE without nephritis, autoimmune/renal diseases and healthy controls.

The mean level was reported to be 3.19 ± 2.2 ng/ml.

The mean urinary TWEAK level was 3.47 ± 2.18 in SLE without nephritis and 3.26 ± 2.55 in lupus nephritis group. Group II (Autoimmune and Renal) was 3.67 ± 2.44. Surprisingly, the TWEAK levels were highest in the disease control group, closely followed by the SLE and Lupus Nephritis groups. The difference however was not statistically different (One-way ANOVA analysis- $p = 0.888$) suggesting that urinary TWEAK increases in inflammatory conditions but is not specific for SLE patients(both renal and non renal). Kralisch et al (98) found lower levels of serum TWEAK in patients with end stage renal disease (p value <0.05), he did not study autoimmune diseases hence his study cannot be used as a comparison. Similarly serum TWEAK levels were found to be significantly lower in haemodialysis patients as compared to healthy individuals (208 versus 461 pg/ml, $p < 0.0001$) in Carrero et al study(99). Schwartz et al (24) had a finding of lower levels of serum TWEAK in SLE patients than in healthy controls. (15.87 vs 23.56, p value < 0.05).In our study group, TWEAK levels were slightly higher in our SLE patients compared to healthy controls.

Schwartz et al (24) study had also reported that urinary TWEAK levels were equally good as serum TWEAK levels for diagnosis of lupus nephritis. Hence, though in our study urinary TWEAK rather than serum TWEAK was analysed, our results can be compared with Schwartz et al.

Contrary to our hypothesis, we found urinary TWEAK levels to be lower in lupus nephritis patients than in SLE without nephritis patients. Further, it was even lower than those with other autoimmune/ renal disease. The reason for this difference from Schwartz study is unclear.

Schwartz study, the only study with a similar methodology as ours reported urinary TWEAK to correlate well with Lupus Nephritis. Our study is not able to corroborate this result. No other study is available in literature for comparison. No data is available for children. Hence we may postulate that further studies are required to test the validity of this marker as a screening test in Lupus Nephritis, and more so in children.

TWEAK VERSUS RENAL SLEDAI

Correlation of urinary TWEAK levels with renal SLEDAI score showed that a higher renal SLEDAI score had a higher median value of urinary TWEAK. Though the values were not statistically significant ($p = 0.743$) it may be suggested that Urinary TWEAK could be a marker for activity in Lupus Nephritis, just as shown by Xueling et al and Schwartz et al study(24) who were able to show a positive correlation between renal SLEDAI score and urinary TWEAK levels ($p < 0.001$).

In our study we were able to recruit 38 children with Lupus Nephritis. Hence a study with as larger sample size, having adequate number of children with active and inactive disease needs to be done in order to see whether it is a good differentiating test for these conditions.

COMPARISON OF URINARY TWEAK LEVELS BETWEEN LUPUS NEPHRITIS CLASSES

The urinary TWEAK levels were compared with different classes of lupus nephritis with the assumption that there will be a positive correlation between the two variables since more renal inflammation and damage is associated with higher class of lupus nephritis. In our study, it was seen that the maximum levels of TWEAK levels were seen in Class III followed by Class II and much lower in IV lupus nephritis. Though the difference was not statistically significant (p – 0.489), it goes against our proposed hypothesis that TWEAK is involved in inflammatory responses especially in renal tissues. It is possible that the unequal distribution of patients in each class of lupus nephritis and the overall small number of patients could not give us a significant correlation. Schwartz et al study (24), urinary TWEAK level was also not able to discriminate between the histological class of lupus nephritis.

CORRELATION BETWEEN URINARY TWEAK LEVELS AND ANTI dsDNA ANTIBODIES

Urinary TWEAK levels were compared against the currently available parameters used to assess the disease activity.

A Pearson's Correlation was run to assess the correlation between Urinary TWEAK levels and ds DNA antibodies. There was positive correlation between urinary TWEAK levels and anti ds DNA antibodies but the correlation was not found to be statistically significant (p = 0.686). The correlation coefficient with urine protein creatinine ratio

was 0.2 indicating a positive correlation with renal involvement. However the correlation was not statistically significant (p= 0.228).

A small negative correlation was found between serum creatinine levels and urinary TWEAK levels, however the correlation was not statistically significant (p= 0.234, r = -0.19). There was a negative correlation between TWEAK levels and both complement C3 and C4 levels (r = -0.21 and -0.06 respectively). However, the correlation among these two levels was also not strong.

Thus, when used alone, urinary TWEAK level showed a positive correlation with anti ds DNA and Urine Protein Creatinine ratio and a negative correlation with Serum Creatinine and C3 levels. This suggests that TWEAK would be a better screening test rather than a diagnostic Test. It is no better than Ds DNA and C 3, C4 levels and Urine Protein Creatinine ratio which are the current tests in use. The negative correlation with creatinine suggests that TWEAK levels may start declining once renal function starts worsening.

A ROC curve was plotted for determining the sensitivity and specificity of Urinary TWEAK as a diagnostic test.The area under the curve was 0.46.The sensitivity of TWEAK for determining the disease activity was 60.53% and the specificity was found to be 36.11%.The cut off value for determining the active disease was found to be more than 2.7

Further studies on this Biomarker may be undertaken to give a clearer picture about the suitability of TWEAK as a biomarker for SLE patients.

SUMMARY

1) A total of 156 children who fulfilled the inclusion criteria were recruited for tis study (40 in lupus nephritis, 36 in SLE, 40 in disease control and 40 in healthy children group)

2) The median age for lupus nephritis group and SLE without nephritis group was 14 whereas it was 12.5 in the disease control group (other autoimmune and renal disease) and 8 in healthy controls.

3) Both lupus nephritis (Ia) and SLE without nephritis (Ib) groups had female preponderance with male : female ratio of 1 : 3.4 in group Ia and 1 : 2.6 ratio in group Ib .

4) Both Ia and Ib groups had higher body mass index of 19 and 18.4 respectively as compared to disease control and healthy control children.

5) Median duration of disease in group Ia patients was 24 months and 17 months in group Ib.

6) Both group Ia and Ib patients had similar clinical features. ANA was positive in all SLE patients (100%) as compared to 85% among lupus nephritis patients. Immune disorder as characterized by elevated anti ds DNA were in 87.5% and 88.9% of patients with Group Ia and Group Ib respectively. Common non renal manifestations were musculoskeletal (Group Ia 63.9% and Group Ib 70%) followed by haematological(57.5% in Group Ia and 50% in Group Ib) and

mucocutaneous involvement. Other rarer manifestations include serositis, neurological involvement and discoid rash.

7) Among the renal parameters in group Ia, majority had proteinuria - 29 patients (72.5%) followed by 24 patients (60%) each with haematuria and pyuria. Only 6 patients (15%) had urinary casts.

8) 13 patients (32.5%) had renal SLEDAI score of twelve followed by 8 patients (20%) with renal SLEDAI score of four. Renal SLEDAI score was not available for one patient

9) Of the total, 65% (26/36) of the patients had Class IV Lupus nephritis (Diffuse proliferative GN) followed by Class III Lupus (Focal proliferative GN) nephritis in 12.5% (5/36)

10) Class IV lupus nephritis were accounted for most of the cases of nephrotic range proteinuria (61.5%), haematuria (71.4%), hypertension (71.4%), pyuria (68.4%) and urinary casts (50%).

11) Among laboratory parameters only complement levels C3 and C4 were significantly assocaited with the newly diagnosed, relapse and remission patients in group Ia (p value < 0.05).

12) Higher percentage of patients with relapse and remission were on mycophenolate mofetil as compared to newly diagnosed lupus nephritis. Most newly diagnosed patients were on prednisolone.

13) Among the laboratory parameters, complement C3 levels, urine protein creatinine ratio and serum creatinine levels showed significant difference between Group I a and Group Ib patients with p value < 0.05.

14) Systolic and diastolic blood pressure was found to be higher among Group Ia patients (p value < 0.05)

15) 30 patients 75% of the patients (30/40) with lupus nephritis were on corticosteroid as compared to 55.5% of SLE patients (20/36). The most commonly used second line drug in both groups was hydroxychloroquine. Mycophenolate mofetil were more commonly used among lupus nephritis as compared to SLE patients (45% versus 5.5%).

16) 134 children from four different groups (38 in lupus nephritis, 36 in SLE without nephritis, 40 in healthy control and 21 in disease control) were tested for urinary TWEAK levels.

17) The mean level of Urinary Tweak levels was 3.49 ± 2.29 ng/ml, ranging from 0.02 to 10.35 ng/ml

18) The Urinary TWEAK levels were highest in the SLE without Nephritis group, closely followed by the Autoimmune /Renal disease group. The SLE Nephritis group had lower values, but was higher than the Healthy Controls. However, the difference between these groups was not statistically significant. (p value = 0.888).

19) Higher renal SLEDAI score had a higher median value of urinary TWEAK. However the values were not statistically significant (p value 0.174).

20) Maximum levels of TWEAK levels were seen in Class III followed by Class II and then by IV lupus nephritis. The difference was not statistically significant (p = 0.174).

21) There was mild positive correlation between TWEAK levels and anti ds DNA, and urine protein creatinine ratio. The difference was not statistically significant($p > 0.05$)

22) There was negative correlation between urinary TWEAK levels and serum creatinine and complement C3 and C4 levels but it was not statistically significant ($p > 0.05$)

23) The ROC plot showed an Area under the curve of 0.46. The sensitivity of TWEAK for determining the disease activity was 60.53% and the specificty was found to be 36.11%.

24) The cut off value for determining the active disease was > 2.7 ng/ml.

CONCLUSIONS

1. Urinary TWEAK levels among paediatric age group could not differentiate lupus nephritis patients from SLE patients without renal involvement.

2. Urinary TWEAK levels were also elevated in children with other Autoimmune /renal diseases hence could not differentiate between SLE and non SLE patients.

3. Urinary TWEAK had a positive correlation with renal SLEDAI score hence may be considered to show promise as a marker of activity in Lupus Nephritis.

3. The TWEAK levels were not able to differentiate between the different Classes of Lupus Nephritis.

4.Urinary TWEAK had a sensitivity of 60.53% and specificty of 36.11% for diagnosing Lupus Nephritis among SLE patients. Hence it may be a better screening test rather than a diagnostic test.

LIMITATIONS

1. Majority of the patients in lupus nephritis group were already on corticosteroids or second line of immunomodulators. Whether prior doses of steroids significantly reduce the levels of urinary TWEAK is not known.

2. Larger and longitudinal studies would be helpful to know the progression of urinary TWEAK over time in relation to its renal disease in lupus nephritis.

3. Urinary TWEAK levels were lower than expected in the disease group (lupus nephritis and SLE without nephritis) as compared to other autoimmune and renal disease group. Whether this was due to timing of specimen collection is not known.

4. Whether healthy controls in general have relatively higher levels of urinary TWEAK is not known. This finding needs further evaluation.

RECOMMENDATIONS

Further studies among paediatric population with larger sample size of Lupus Nephritis patients are needed to re-evaluate the usefulness of urinary TWEAK as a test.

Other new emerging biomarkers for diagnosis of lupus nephritis need to be studied to assess their correlation with disease activity and class of lupus nephritis.

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ANNEXURES

1……SLEDAI SCORE

2…….PATIENT INFORMATION

SHEET/INFORMED CONSENT FORMS

3…….CASE REPORT FORM

4……. TWEAK ASSAY PROCEDURE

5……. DATA EXCEL SPREADSHEET

ANNEXURE I

SLE DISEASE ACTIVITY INDEX SCORE

Table 7

SLE Daily Activity Index: Data Collection Sheet

SLEDAI Score	Descriptor	Definition
8	Seizures	Recent onset. Exclude metabolic, infectious or drug causes.
8	Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized or catatonic behavior. Exclude uremia and drug causes.
8	Organic brain syndrome	Altered mental function with impaired orientation, memory, or other intellectual function, with rapid onset and fluctuating clinical features. Include clouding of consciousness with reduced capacity to focus, and inability to sustain attention to environment, plus at least 2 of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness or increased or decreased psychomotor activity. Exclude metabolic, infection or drug causes.
8	Visual disturbance	Retinal changes of SLE. Include cytoid bodies, retinal hemorrhages, serous exudate or hemorrhages in the choroid or optic neuritis. Exclude hypertension, infection or drug causes.
8	Cranial nerve disorder	New onset of sensory or motor neuropathy involving cranial nerves.
8	Lupus headache	Severe, persistent headache: may be migrainous, but must be nonresponsive to narcotic analgesia.
8	CVA	New onset of cerebrovascular accident(s). Exclude arteriosclerosis.
8	Vasculitis	Ulceration, gangrene, tender finger nodules, periungual infraction, splinter hemorrhages, or biopsy or angiogram proof of vasculitis.
4	Arthritis	More than 2 joints with pain and signs of inflammation (i.e., tenderness, swelling or effusion).
4	Myositis	Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/aldolase or electromyogram changes or a biopsy showing myositis.
4	Urinary casts	Heme-granular or red blood cell casts.
4	Hematuria	>5 red blood cells high power field. Exclude stone, infection or other cause.
4	Proteinuria	>0.5 gm/24 hours. New onset or recent increase of more than 0.5 gm/24 hours.
4	Pyuria	>5 white blood cells/high power field. Exclude infection.
2	New rash	New onset or recurrence of inflammatory type rash.
2	Alopecia	New onset or recurrence of abnormal, patchy or diffuse loss of hair.
2	Mucosal ulcers	New onset or recurrence of oral or nasal ulcerations.
2	Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening.
2	Pericarditis	Pericardial pain with at least 1 of the following: rub, effusion or electrocardiogram or echocardiogram confirmation.
2	Low complement	Decrease in CH50, C3 or C4 below the lower limit of normal for testing laboratory.
2	Increased DNA binding	>25% binding by Farr assay or above normal range for testing laboratory.
1	Fever	>38°C. Exclude infectious cause.
1	Thrombocytopenia	<100,000 platelets/mm^3.
1	Leukopenia	<3,000 white blood cells/mm^3. Exclude drug causes.

TOTAL SLEDAI SCORE: _____

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ANNEXURE II

PARTICIPANT INFORMATION SHEET

Christian Medical College, Vellore

Department of Child Health II

Role of urinary TWEAK as a biomarker in children with Lupus Nephritis

You are being requested to allow your child to participate in a study to evaluate the effectiveness of urinary TWEAK, a new test for involvement of kidney in lupus nephritis. Studies have shown that this new test is a better marker than the standard test available.Urine sample for all children with suspected SLE will be collected and analyzed. Results will be correlated with the standard test which suggests kidney involvement. We will also check this marker in normal children as well as children with other forms of kidney disease and autoimmune diseases. If we find increase in level of TWEAK in SLE children with kidney involvement, it will serve as an early marker and help in early intervention. Participation in this study involves giving urine specimen for this study and blood sample for future studies (optional) both of which are easy test to perform without any complications.All details including personal data and assessment of the doctor will be kept confidential. Participation is purely voluntary, and you can withdraw your child from the study at any time and that refusal to participate will not involve any penalty or loss of benefits to which your child is otherwise entitled.

In case of doubts/questions, please contact Dr Muniya Thokchom, Department of Child Health, CMCH, Vellore, phone no. 9597863326

INFORMED ASSENT FORM

Christian Medical College, Vellore

Department of Child Health II

Role of urinary TWEAK as a biomarker in children with Lupus Nephritis

STUDY NUMBER: NAME:

AGE: SEX:

PHONE NUMBER: ADDRESS:

1) I confirm that I have read and understood the information sheet for the above the study and have had the opportunity to ask questions.
2) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.
3) I understand that the Principal investigator, the Ethics committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However I understand that my identity will not be revealed in any information released to the third parties or published.
4) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purposes.

5) I understand that this study involves only urine specimen but blood specimen will also be collected simultaneously which may be used for related studies later in future.

a) I am willing to give both urine and blood specimen for this study ☐

OR

I am willing to give only urine sample for this study ☐

6) I agree to take part in the above study.

Signature of the subject:

Signatory's name: Date:

Thumb impression: ☐

Signature of the parent/legally acceptable representative:

Signatory's name: Date:

Thumb impression: ☐

Signature of the Investigator:

Study Investigator's name: Date:

INFORMED CONSENT FORM

Christian Medical College, Vellore

Department of Child Health II

Role of urinary TWEAK as a biomarker in children with Lupus Nephritis

STUDY NUMBER: NAME:

AGE: SEX:

PHONE NUMBER: ADDRESS:

1) I confirm that I have read and understood the information sheet for the above the study and have had the opportunity to ask questions.
2) I understand that the participation of my child in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my child's medical care or legal rights being affected.
3) I understand that the Principal investigator, the Ethics committee and the regulatory authorities will not need my permission to look at my child's health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw my child from the trial. I agree to this access. However I understand that my child's identity will not be revealed in any information released to the third parties or published.
4) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purposes.
5) I understand that this study involves only urine specimen but blood specimen will also be collected simultaneously which may be used for related studies later in future.

a) I am willing to let my child give both urine and blood specimen for this study

OR

b) I am willing to let my child give only urine sample for this study

6) I agree to let my child take part in the above study.

Signature of the parent/legally acceptable representative:

Signatory's name: Date:

Thumb impression:

Signature of the Investigator:

Study Investigator's name: Date:

Signature of the witness:

Witness' name: Date:

Thumb impression:

ANNEXURE III

CASE REPORT FORM

Role of urinary TWEAK as a biomarker in children with Lupus Nephritis

Serial number: Date

Name: Hospital number Age Sex

Height: Weight: BMI

Phone number: Address

Group :

Lupus nephritis (Ia)	SLE with LN (Ib)	Autoimmune /other renal diseases	Healthy controls

Disease duration Renal biopsy (WHO classification) :

1982 ACR criteria for SLE

Malar rash		Renal disorder	
Discoid rash		Neurological disorder	
Photosensitivity		Hematological disorder	
Oral ulcers		Immune disorder	
Non erosive arthritis		Positive ANA	
Serositis			

SLEDAI SCORE: TOTAL SCORE:

8	SEIZURES	
8	PSYCHOSIS	
8	ORGANIC BRAIN SYNDROME	
8	VISUAL DISTURBANCE	
8	CRANIAL NERVE DISORDER	
8	LUPUS HEADACHE	
8	CEREBROVASCULAR ACCIDENTS	
8	VASCULITIS	
4	ARTHRITIS	
4	MYOSITIS	
4	URINARY CASTS	
4	HAEMATURIA	
4	PROTEINURIA	
4	PYURIA	
2	NEW RASH	

2	ALOPECIA	
2	MUCOSAL ULCERS	
2	PLEURISY	
2	PERICARDITIS2	
2	LOW COMPLEMENT	
2	INCREASED DNA BINDING	
1	FEVER	
1	THROMBOCYTOPENIA	
1	LEUKOPENIA	

Renal SLEDAI SCORE: TOTAL SCORE:

4	URINARY CASTS	
4	HAEMATURIA	
4	PROTEINURIA	
4	PYURIA	

EXAMINATION: Blood pressure: 95^{th} centile for the age/sex :

INVESTIGATIONS:

Urine routine and microscopy	Anti ds DNA antibodies	UP/UC	Urine TWEAK	Renal biopsy
RBCs :				
WBCs :				
Proteinuria:				
Casts :				

Haemoglobin	WBC	Platelets	Creatinine	C3 C4 levels

TREATMENT:

Drug	Duration
Steroid :	
Immunomodulator :	

ANNEXURE IV

Human TWEAK ELISA kit

ASSAY PROCEDURE

1) 100 microL of sample or standard was added to the appropriate number of wells in the supplied Neoplate. 100microL of PBS (pH 7.0 – 7.2) to the blank well was added
2) 50 microL of enzyme solution to each well in the supplied neoplate was added and mixed well
3) Neoplate was covered and incubated for 1 hour at 37'C in a humid chamber
4) Each well is washed 5 times with 300 – 400 microL 1X wash well per well. After the last wash the plates is inverted and blot dry by tapping on absorbent paper.
5) 50 microL of substrate A was added to each well followed by addition of 50 microL of substrate B. It is then covered and incubated 10 -15 minutes at room temperature.
6) 50 microL of stop solution is added to each well and mixed well
7) Optical density (O.D) is read immediately at 450nm
8) The mean blank value from each sample or standard value is subtracted and the mean for duplicate wells is calculated
9) The standard curve is constructed using graph paper or statistical software

sno	date	age	sex	ht	wt	bmi	grp	ddur	rbiop	malar	dis	photosens	oral	arthritis	serositis	renal	neuro	haemat	immune	ana	acr	sledai
2	1/23/2015	18	1	179	85	26.6	1	60	4	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	1	0
3	7/30/2015	10	2	140	38	19.3	1	6	4	FALSE	FALSE	FALSE	TRUE	TRUE	FALSE	TRUE	FALSE	FALSE	TRUE	TRUE	5	23
4	7/29/2015	14	1	154	45	19.2	1	30	4	TRUE	FALSE	FALSE	TRUE	TRUE	FALSE	TRUE	FALSE	FALSE	TRUE	TRUE	6	24
5	7/20/2015	15	2	162	84	32.1	1	36	4	TRUE	FALSE	FALSE	TRUE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	6	25
6	7/17/2015	8	2	109	18	15.8	1	12	0	TRUE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	6	35
7	7/10/2015	14	2	149	48	21.5	1	24	4	TRUE	FALSE	FALSE	TRUE	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	7	20
8	7/17/2015	14	2	158	38	15.3	1	7	2	TRUE	TRUE	FALSE	TRUE	TRUE	TRUE	TRUE	FALSE	TRUE	TRUE	TRUE	9	29
9	7/10/2015	14	2	151	50	21.8	1	15	3	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	TRUE	TRUE	4	24
10	7/3/2015	13	2	142	31	15.4	1	1	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	4	12
11	5/1/2015	13	1	144	31	14.9	1	21	4	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	1	14
12	7/8/2015	12	2	138	39	20.5	1	2	4	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	4	22
13	12/24/2014	16	2	158	47	18.8	1	35	3	TRUE	FALSE	FALSE	TRUE	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	7	26
14	9/2/2015	11	2	143	30	14.8	1	1	4	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	TRUE	TRUE	4	23
15	4/11/2015	13	2	163	49	18.6	1	9	4	TRUE	FALSE	FALSE	TRUE	TRUE	FALSE	TRUE	FALSE	FALSE	TRUE	TRUE	6	28
16	5/6/2015	9.5	2	121	23	16.1	1	3	0	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	FALSE	TRUE	TRUE	TRUE	6	18
17	5/1/2015	14	2	151	40	17.8	1	6	4	TRUE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	FALSE	FALSE	TRUE	TRUE	6	25
18	6/23/2015	12	2	154	49	20.7	1	1	5	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	4	13
19	6/5/2015	13	1	143	30	14.8	1	17	3	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	5	25
20	6/13/2015	18	2	164	60	22.6	1	72	2	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	TRUE	TRUE	4	14
21	5/1/2015	17	2	144	38	18.2	1	48	4	TRUE	FALSE	TRUE	TRUE	TRUE	FALSE	TRUE	FALSE	FALSE	TRUE	TRUE	7	24
22	12/12/2014	16	2	147	34	15.7	1	60	4	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	FALSE	FALSE	TRUE	TRUE	5	17
23	5/6/2015	17	1	152	40	17.5	1	48	4	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	3	18
24	3/6/2015	16	2	155	51	21.1	1	24	3	TRUE	FALSE	FALSE	TRUE	TRUE	FALSE	TRUE	FALSE	FALSE	TRUE	TRUE	6	26
25	12/22/2014	15	2	156	52	21.4	1	24	6	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	4	16
26	4/10/2015	14	2	137	41	22.1	1	27	4	FALSE	FALSE	TRUE	TRUE	TRUE	FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	8	10
27	1/23/2015	9	2	128	23	14	1	36	2	TRUE	FALSE	FALSE	TRUE	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	7	16
28	3/4/2015	12	1	144	42	20.4	1	36	4	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	4	11
29	6/19/2015	15	1	159	58	23.1	1	48	4	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	TRUE	TRUE	4	6
30	6/19/2015	14	2	145	35	16.5	1	22	4	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	TRUE	TRUE	3	4
31	6/26/2015	14	2	146	46	21.8	1	8	4	TRUE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	6	4
32	6/26/2015	9	2	120	24	16.7	1	4	0	FALSE	FALSE	FALSE	TRUE	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	6	8
33	4/29/2015	14	2	144	48	23.1	1	30	4	TRUE	FALSE	TRUE	TRUE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE	TRUE	6	8
34	6/26/2015	11	1	125	26	16.7	1	84	4	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	TRUE	TRUE	3	6
35	6/12/2015	11	2	137	42	22.4	1	17	4	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	1	10
36	6/19/2015	16	2	149	45	20.5	1	17	4	FALSE	FALSE	TRUE	TRUE	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	7	0
37	6/19/2015	7	2	123	25	16.5	1	26	4	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	4	1
38	2/6/2015	13	1	148	36	16.3	1	4	4	FALSE	FALSE	FALSE	TRUE	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	6	4
39	6/24/2015	7	2	144	47	22.7	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
40	6/10/2016	13	2	151	38	16.8	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
41	6/13/2015	10	1	131	26	15.2	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
42	6/10/2015	5	1	111	15	11.8	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
43	6/13/2016	7	2	122	20	13.6	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
44	6/3/2015	12	1	156	45	18.5	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
45	6/24/2015	8	2	133	26	15	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
46	6/20/2015	9	2	115	16	12.1	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
47	6/17/2015	16	2	157	48	19.7	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
48	6/6/2015	15	2	154	42	17.8	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
49	6/20/2015	11	1	145	28	13.2	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
50	6/20/2015	11	1	143	52	25.6	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
51	6/27/2015	6	1	100	13	13	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
52	6/10/2015	7	2	115	18	14.1	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
53	6/10/2015	6	1	119	20	14.3	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
54	6/10/2015	5	1	116	19	14.4	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
55	6/17/2015	13	1	147	32	14.9	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		

56	6/6/2015	8	1	135	31	17.3	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
57	6/6/2015	10	1	112	15	12.9	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
58	6/20/2015	13	1	148	53	24.5	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
59	6/6/2015	6	1	107	14	11.9	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
60	6/20/2015	7	1	120	21	14.6	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
61	6/24/2015	14	1	177	80	25.7	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
62	6/6/2015	10	1	131	26	15.2	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
63	6/27/2015	5	1	114	20	15.4	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
65	6/13/2015	15	1	165	45	16.8	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
66	6/27/2015	4	1	104	13	12.4	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
67	6/6/2015	8	1	133	23	13	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
68	6/20/2015	12	1	139	26	13.2	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
69	9/4/2015	9	2	128	27	16.6	2	22	0	TRUE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	TRUE	4	10
70	6/26/2015	15	2	151	43	18.9	2	11	0	TRUE	FALSE	TRUE	TRUE	TRUE	FALSE	TRUE	FALSE	FALSE	TRUE	TRUE	7	16
71	5/1/2015	10	1	126	20	12.8	2	6	0	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	4	4
72	6/26/2015	16	2	152	40	17.3	2	18	0	TRUE	FALSE	TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	7	15
73	2/20/2015	14	2	155	44	18.4	2	2	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	2	14
74	4/10/2015	13	2	150	49	21.7	2	4	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	2	0
75	6/19/2015	16	1	156	39	16	2	28	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	3	4
76	12/24/2014	16	2	153	47	20.2	2	10	0	TRUE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	6	9
77	6/12/2015	12	2	141	42	21.1	2	8	0	TRUE	FALSE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	5	12
78	1/23/2015	13	1	130	22	13.1	2	6	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	2	3
79	6/19/2015	18	2	154	50	21.3	1	74	4	TRUE	FALSE	FALSE	TRUE	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	7	0
80	6/19/2015	18	1	55	96	40	2	91	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	2	0
81	6/20/2015	7	1	113	15	12.4	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
82	6/10/2015	7	1	116	17	13.2	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
83	7/13/2015	6	2	110	14	11.6	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
84	7/13/2015	9	1	111	17	13.6	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
85	8/1/2015	7	2	100	14	14	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
86	8/1/2015	5	1	117	16	11.7	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
87	8/1/2015	7	2	118	16	11.5	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
88	8/1/2015	9	1	128	21	12.8	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
89	8/1/2015	8.5	1	135	39	21.4	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
90	8/1/2015	5.6	2	112	17	13.6	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
91	8/1/2015	7	2	122	24	16.1	4	0	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
92	7/23/2015	17	2	150	84	37.3	2	40	0	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	4	12
93	7/14/2015	12	1	138	25	13.1	2	3	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	3	7
94	8/5/2015	14	2	145	32	15.2	2	17	0	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	4	6
95	8/7/2015	11	2	138	38	20	2	6	0	TRUE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	5	6
96	8/1/2015	16	2	149	40	18	2	17	0	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	4	14
97	1/23/2015	11	2	127	23	13.9	2	38	0	TRUE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	TRUE	TRUE	TRUE	6	2
98	2/20/2015	15	1	156	45	18.7	2	18	0	TRUE	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	5	0
99	12/19/2014	16	2	142	35	17.2	2	51	0	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	4	13
100	2/20/2015	13	2	151	49	21.7	2	7	0	TRUE	FALSE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	5	4
101	4/10/2015	9	1	133	26	14.8	2	3	0	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	3	3
102	6/13/2015	15	2	147	42	24.9	2	24	0	TRUE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	4	4
103	4/10/2015	15	2	164	57	21.3	2	8	0	TRUE	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	5	18
104	6/5/2015	15	1	161	53	20.4	2	8	0	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	4	0
105	6/17/2015	9	2	122	26	17.5	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
106	6/26/2015	5	1	99	16	16.3	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
107	2/6/2015	15	2	162	36	14.7	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
108	1/23/2015	15	2	145	31	14.7	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
109	2/6/2015	13	2	125	27	17.3	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
110	12/19/2014	15	1	152	41	17.7	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
111	6/3/2015	4	1	95	14	15.5	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		

112	6/13/2015	14	2	158	40	16	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
113	6/3/2015	7	1	121	25	17.1	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
114	6/19/2015	12	2	149	31	14	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
115	2/6/2015	11	1	136	25	13.5	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
116	4/10/2015	17	1	175	59	19.3	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
117	6/19/2015	6	2	106	12	10.7	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
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119	1/23/2015	16	1	154	36	15.2	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
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121	12/5/2014	8	1	122	24	16.1	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
122	6/17/2015	16	1	137	34	18.1	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
123	6/5/2015	14	1	140	35	17.9	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
124	6/19/2015	6	1	110	18	14.9	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
125	4/10/2015	17	1	163	56	21.1	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
126	12/19/2014	12	1	132	23	13.2	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
127	12/19/2014	10	1	126	21	13.2	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
128	12/19/2014	12	1	127	25	15.5	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
129	4/10/2015	5	1	100	15	15	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
130	6/17/2015	17	1	143	31	15.2	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
131	6/3/2015	5	1	104	18	16.6	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
132	4/10/2015	6	1	117	22	16.1	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
133	3/6/2015	6	2	110	16	13.2	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
134	2/20/2015	13	1	134	23	12.8	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
135	4/10/2015	15	1	154	30	12.6	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
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137	6/19/2015	5	1	106	16	14.2	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
138	3/6/2015	15	2	155	57	23.7	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
139	6/3/2015	14	1	153	48	20.5	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
140	1/23/2015	13	2	149	44	19.8	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
141	2/20/2015	16	1	169	52	18.2	3		0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
142	7/3/2015	14	2	146	33	15.4	2	26	0	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	TRUE	TRUE	5	0
143	6/13/2015	11	2	135	29	15.9	2	52	0	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	4	0
144	9/11/2015	14	2	152	42	18.4	2	3	0	TRUE	FALSE	TRUE	TRUE	TRUE	FALSE	TRUE	TRUE	FALSE	TRUE	TRUE	8	14
145	7/3/2015	10	2	147	41	18.9	2	23	0	TRUE	FALSE	TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	6	4
146	7/3/2015	12	2	155	58	24	2	22	0	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	TRUE	TRUE	5	16
147	7/31/2015	15	2	152	59	25.4	2	18	0	TRUE	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	5	12
148	8/7/2015	18	2	153	40	17.1	2	79	0	TRUE	FALSE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	6	4
149	8/7/2015	18	2	150	43	18.9	2	54	0	TRUE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	4	6
150	4/10/2015	12	1	145	38	18.3	2	3	0	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	4	2
151	6/13/2015	15	2	149	40	18	2	3	0	TRUE	FALSE	FALSE	TRUE	TRUE	FALSE	FALSE	TRUE	TRUE	TRUE	TRUE	7	26
152	6/12/2015	17	2	156	62	25.7	2	46	0	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	4	5
153	2/20/2015	14	1	156	39	16	3	2	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
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155	3/6/2015	14	1	151	51	22.4	3	61	0	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
157	6/5/2015	17	2	158	40	16	1	34	4	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	4	3
158	9/4/2015	12	1	140	27	13.8	2	3	0	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	5	9
159	8/5/2015	13	2	145	35	26	1	30	3	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	6	0

ucast	hemat	pro	pyu	rsledai	sbp	dbp	sbpcent95	dbpcent95	urbc	uwbc	protein	casts	dsdna	upuc	utweak	hb	wbc	plt	crt	c3	c4	strds
2	2	2	2	0	110	80	134	82	0	0	0	FALSE	3	0.06		13.7	7700	306000	0.7	123	20.2	0
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2	2	1	1	8	113	76	124	81	3	12	2	FALSE	233	0.84		5.8	8300	111000	0.6	17.7	6.2	30
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2	2	1	2	4	140	80	135	85	2	1	0	FALSE	284	1.43		15.7	9700	173000	0.5	81.1	15.7	0
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2	1	2	2	4	109	67	129	84	11	2	0	FALSE	14	0.02		12	11500	179000	0.8	105	12.1	15
2	1	2	2	4	100	70	120	79	6	4	0	FALSE	140	0.14		12	8700	304000	0.4	79	6.3	7.5
9	9	9	9		122	60	129	84				FALSE		0.56		12.2	11100	241000	0.3	132	25.8	5
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dur	immuno	mmf	hcq	azathio	cyclo	others
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